

FILE 'HOME' ENTERED AT 16:00:51 ON 20 NOV 2003

=> file biosis,caba,caplus,embase,japio,lifesci,medline,scisearch,uspatfull

=> e levinson douglas/au

E1 1 LEVINSON DONALD B/AU
E2 6 LEVINSON DOUG/AU
E3 19 --> LEVINSON DOUGLAS/AU
E4 12 LEVINSON DOUGLAS A/AU
E5 38 LEVINSON DOUGLAS ADAM/AU
E6 51 LEVINSON DOUGLAS F/AU
E7 3 LEVINSON DUSHNIK M/AU
E8 2 LEVINSON DUSHNIK MATAT/AU
E9 66 LEVINSON E/AU
E10 3 LEVINSON E A/AU
E11 59 LEVINSON E D/AU
E12 8 LEVINSON E E/AU

=> s e2-e5

L1 75 ("LEVINSON DOUG"/AU OR "LEVINSON DOUGLAS"/AU OR "LEVINSON DOUGLA
S A"/AU OR "LEVINSON DOUGLAS ADAM"/AU)

=> dup rem l1

PROCESSING COMPLETED FOR L1

L2 50 DUP REM L1 (25 DUPLICATES REMOVED)

=> s l2 and antibod?

L3 13 L2 AND ANTIBOD?

=> d bib ab kwic 1-

YOU HAVE REQUESTED DATA FROM 13 ANSWERS - CONTINUE? Y/(N):y

L3 ANSWER 1 OF 13 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN

AN 2001:355560 BIOSIS

DN PREV200100355560

TI TH2-specific gene.

AU ***Levinson, Doug*** [Inventor, Reprint author]; Gu, Wei [Inventor];
Lehar, Sophie [Inventor]

CS Sherborn, MA, USA

ASSIGNEE: Millennium Pharmaceuticals, Inc.

PI US 6190909 February 20, 2001

SO Official Gazette of the United States Patent and Trademark Office Patents,
(Feb. 20, 2001) Vol. 1243, No. 3. e-file.

CODEN: OGUPE7. ISSN: 0098-1133.

DT Patent

LA English

ED Entered STN: 2 Aug 2001

Last Updated on STN: 19 Feb 2002

AB The present invention relates to the discovery, identification and
characterization of nucleic acids that encode a novel protein
differentially expressed within the TH2 cell subpopulation (hereinafter
referred to as STIF). The invention encompasses STIF nucleotides, host
cell expression systems, STIF proteins, fusion proteins, polypeptides and
peptides, ***antibodies*** to the STIF protein, transgenic animals
that express a STIF transgene, or recombinant knock-out animals that do
not express the STIF protein, and compounds that modulate STIF gene
expression or STIF activity that can be used for diagnosis, drug

screening, clinical trial monitoring, and/or used to treat STIF based disorders, such as proliferative disorders and T-lymphocyte-related disorders including, but not limited to, chronic inflammatory diseases and disorders, such as Crohn's disease, reactive arthritis, including Lyme disease, insulin-dependent diabetes, organ-specific autoimmunity, including multiple sclerosis, Hashimoto's thyroiditis and Grave's disease, contact dermatitis, psoriasis, graft rejection, graft versus host disease, sarcoidosis, atopic conditions, such as asthma and allergy, including allergic rhinitis, gastrointestinal allergies, including food allergies, eosinophilia, conjunctivitis, glomerular nephritis, certain pathogen susceptibilities such as helminthic (e.g., leishmaniasis) and certain viral infections, including HIV, and bacterial infections, including tuberculosis and lepromatous leprosy.

AU ***Levinson, Doug*** [Inventor, Reprint author]; Gu, Wei [Inventor]; Lehar, Sophie [Inventor]

AB. . . referred to as STIF). The invention encompasses STIF nucleotides, host cell expression systems, STIF proteins, fusion proteins, polypeptides and peptides, ***antibodies*** to the STIF protein, transgenic animals that express a STIF transgene, or recombinant knock-out animals that do not express the. . .

L3 ANSWER 2 OF 13 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN

AN 2000:3188 BIOSIS

DN PREV200000003188

TI Crucial role of the interleukin 1 receptor family member T1/ST2 in T helper cell type 2-mediated lung mucosal immune responses.

AU Coyle, Anthony J. [Reprint author]; Lloyd, Clare [Reprint author]; Tian, Jane [Reprint author]; Nguyen, Trang [Reprint author]; Eriksson, Christina; Wang, Lin [Reprint author]; Ottoson, Par; Persson, Per; Delaney, Tracy [Reprint author]; Lehar, Sophie [Reprint author]; Lin, Steve [Reprint author]; Poisson, Louis [Reprint author]; Meisel, Christian; Kamradt, Thomas; Bjerke, Torbjorn; ***Levinson, Douglas*** [Reprint author]; Gutierrez-Ramos, Jose Carlos [Reprint author]

CS Department of Biology, Inflammation Division, Millennium Pharmaceuticals, Inc., Cambridge, MA, 02139, USA

SO Journal of Experimental Medicine, (Oct. 4, 1999) Vol. 190, No. 7, pp. 895-902. print.

CODEN: JEMEAV. ISSN: 0022-1007.

DT Article

LA English

ED Entered STN: 23 Dec 1999

Last Updated on STN: 31 Dec 2001

AB T1/ST2 is an orphan receptor of unknown function that is expressed on the surface of murine T helper cell type 2 (Th2), but not Th1 effector cells. In vitro blockade of T1/ST2 signaling with an immunoglobulin (Ig) fusion protein suppresses both differentiation to and activation of Th2, but not Th1 effector populations. In a nascent Th2-dominated response, anti-T1/ST2 monoclonal ***antibody*** (mAb) inhibited eosinophil infiltration, interleukin 5 secretion, and IgE production. To determine if these effects were mediated by a direct effect on Th2 cells, we next used a murine adoptive transfer model of Th1- and Th2-mediated lung mucosal immune responses. Administration of either T1/ST2 mAb or T1/ST2-Ig abrogated Th2 cytokine production in vivo and the induction of an eosinophilic inflammatory response, but failed to modify Th1-mediated inflammation. Taken together, our data demonstrate an important role of

T1/ST2 in Th2-mediated inflammatory responses and suggest that T1/ST2 may prove to be a novel target for the selective suppression of Th2 immune responses.

AU. . . [Reprint author]; Lehar, Sophie [Reprint author]; Lin, Steve [Reprint author]; Poisson, Louis [Reprint author]; Meisel, Christian; Kamradt, Thomas; Bjerke, Torbjorn; ***Levinson, Douglas*** [Reprint author]; Gutierrez-Ramos, Jose Carlos [Reprint author]

AB. . . suppresses both differentiation to and activation of Th2, but not Th1 effector populations. In a nascent Th2-dominated response, anti-T1/ST2 monoclonal ***antibody*** (mAb) inhibited eosinophil infiltration, interleukin 5 secretion, and IgE production. To determine if these effects were mediated by a direct. . .

L3 ANSWER 3 OF 13 CAPLUS COPYRIGHT 2003 ACS on STN

AN 2000:861842 CAPLUS

DN 134:28454

TI T helper cell-expressed polynucleotide and polypeptide compositions and methods for the treatment and diagnosis of immune disorders

IN ***Levinson, Douglas A.*** ; Lloyd, Clare M.; McCarthy, Sean A.

PA Millennium Pharmaceuticals, Inc., USA

SO PCT Int. Appl., 311 pp.

CODEN: PIXXD2

DT Patent

LA English

FAN.CNT 5

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
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PI WO 2000073498	A1	20001207	WO 2000-US14986	20000531
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W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRAI US 1999-324986 A 19990602

AB The present invention relates to methods and compns. for the treatment and diagnosis of immune disorders, esp. T helper lymphocyte-related disorders, and also for the treatment of mast cell-related processes and disorders, ischemic disorders and injuries, including ischemic renal disorders and injuries. For example, genes which are differentially expressed within and among T helper (TH) cells and TH cell subpopulations, which include, but are not limited to TH0, TH1 and TH2 cell subpopulations are identified. Genes are also identified via the ability of their gene products to interact with gene products involved in the differentiation, maintenance and effector function of such TH cells and TH cell subpopulations. The genes identified can be used diagnostically or as targets for therapeutic intervention. In this regard, the present invention provides methods for the identification and therapeutic use of compds. as treatments of immune disorders, esp. TH cell subpopulation-related disorders. Addnl., methods are provided for the diagnostic evaluation and prognosis of TH cell subpopulation-related disorders, for the identification of subjects exhibiting a predisposition

to such conditions, for monitoring patients undergoing clin. evaluation for the treatment of such disorders, and for monitoring the efficacy of compds. used in clin. trials. Methods are also provided for the treatment of symptoms assocd. with mast cell-related processes or disorders and ischemic disorders and injuries using the genes, gene products and ***antibodies*** of the invention.

RE.CNT 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

IN ***Levinson, Douglas A.*** ; Lloyd, Clare M.; McCarthy, Sean A.

AB The present invention relates to methods and compns. for the treatment and diagnosis of immune disorders, esp. T helper lymphocyte-related disorders, and also for the treatment of mast cell-related processes and disorders, ischemic disorders and injuries, including ischemic renal disorders and injuries. For example, genes which are differentially expressed within and among T helper (TH) cells and TH cell subpopulations, which include, but are not limited to TH0, TH1 and TH2 cell subpopulations are identified. Genes are also identified via the ability of their gene products to interact with gene products involved in the differentiation, maintenance and effector function of such TH cells and TH cell subpopulations. The genes identified can be used diagnostically or as targets for therapeutic intervention. In this regard, the present invention provides methods for the identification and therapeutic use of compds. as treatments of immune disorders, esp. TH cell subpopulation-related disorders. Addnl., methods are provided for the diagnostic evaluation and prognosis of TH cell subpopulation-related disorders, for the identification of subjects exhibiting a predisposition to such conditions, for monitoring patients undergoing clin. evaluation for the treatment of such disorders, and for monitoring the efficacy of compds. used in clin. trials. Methods are also provided for the treatment of symptoms assocd. with mast cell-related processes or disorders and ischemic disorders and injuries using the genes, gene products and ***antibodies*** of the invention.

L3 ANSWER 4 OF 13 CAPLUS COPYRIGHT 2003 ACS on STN

AN 1998:708851 CAPLUS

DN 129:314991

TI Th2-specific gene STIF and diagnosis and treatment of proliferative and immune disorders

IN ***Levinson, Doug*** ; Gu, Wei; Lehar, Sophie

PA Millennium Pharmaceuticals, Inc., USA

SO PCT Int. Appl., 126 pp.

CODEN: PIXXD2

DT Patent

LA English

FAN.CNT 2

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
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PI WO 9846638	A1	19981022	WO 1998-US7616	19980417
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W: AU, CA, JP

RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE

US 6190909	B1	20010220	US 1997-884077	19970625
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AU 9871228	A1	19981111	AU 1998-71228	19980417
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AU 738710	B2	20010927		
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EP 977777	A1	20000209	EP 1998-918268	19980417
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R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
IE, FI

JP 2002514918 T2 20020521 JP 1998-544274 19980417

PRAI US 1997-841901 A 19970417

US 1997-884077 A 19970625

WO 1998-US7616 W 19980417

AB The present invention relates to the discovery, identification and characterization of nucleic acids that encode novel protein STIF differentially expressed within the TH2 cell subpopulation. The invention encompasses STIF nucleotides, host cell expression systems, STIF proteins, fusion proteins, polypeptides and peptides, ***antibodies*** to the STIF protein, transgenic animals that express a STIF transgene, or recombinant knock-out animals that do not express the STIF protein, and compds. that modulate STIF gene expression or STIF activity that can be used for diagnosis, drug screening, clin. trial monitoring, and/or used to treat STIF based disorders, such as proliferative disorders and T-lymphocyte-related disorders. Northern blot anal. indicated that STIF mRNA was present only in TH2 cells. The gene was mapped to mouse chromosome 1.

RE.CNT 2 THERE ARE 2 CITED REFERENCES AVAILABLE FOR THIS RECORD

ALL CITATIONS AVAILABLE IN THE RE FORMAT

IN ***Levinson, Doug*** ; Gu, Wei; Lehar, Sophie

AB The present invention relates to the discovery, identification and characterization of nucleic acids that encode novel protein STIF differentially expressed within the TH2 cell subpopulation. The invention encompasses STIF nucleotides, host cell expression systems, STIF proteins, fusion proteins, polypeptides and peptides, ***antibodies*** to the STIF protein, transgenic animals that express a STIF transgene, or recombinant knock-out animals that do not express the STIF protein, and compds. that modulate STIF gene expression or STIF activity that can be used for diagnosis, drug screening, clin. trial monitoring, and/or used to treat STIF based disorders, such as proliferative disorders and T-lymphocyte-related disorders. Northern blot anal. indicated that STIF mRNA was present only in TH2 cells. The gene was mapped to mouse chromosome 1.

IT ***Antibodies***

RL: ARG (Analytical reagent use); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(anti-STIF; th2-specific gene STIF and diagnosis and treatment of proliferative and immune disorders)

L3 ANSWER 5 OF 13 CAPLUS COPYRIGHT 2003 ACS on STN

AN 1998:221096 CAPLUS

DN 128:291149

TI RATH genes and polypeptides and methods for the treatment and diagnosis of immune disorders

IN ***Levinson, Douglas A.*** ; Gimeno, Carlos J.

PA Millennium Pharmaceuticals, Inc., USA

SO PCT Int. Appl., 128 pp.

CODEN: PIXXD2

DT Patent

LA English

FAN.CNT 2

PATENT NO. KIND DATE APPLICATION NO. DATE

PI WO 9814579 A1 19980409 WO 1997-US18259 19971006
W: AL, AM, AU, AZ, BA, BB, BG, BR, BY, CA, CN, CU, CZ, EE, GE, GH,
HU, ID, IL, IS, JP, KG, KP, KR, KZ, LC, LK, LR, LT, LV, MD, MG,
MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, SL, TJ, TM, TR, TT,
UA, UZ, VN, YU, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR,
GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA,
GN, ML, MR, NE, SN, TD, TG
US 5846780 A 19981208 US 1996-726228 19961004
US 6020142 A 20000201 US 1997-870815 19970606
AU 9748133 A1 19980424 AU 1997-48133 19971006
AU 735317 B2 20010705
EP 958361 A1 19991124 EP 1997-910858 19971006
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
IE, FI
JP 2002507113 T2 20020305 JP 1998-516980 19971006
PRAI US 1996-726228 A 19961004
US 1997-870815 A 19970606
WO 1997-US18259 W 19971006

AB The present invention relates, first, to the identification of novel nucleic acid mols., termed RATH genes and RATH gene products encoded by such nucleic acid mols., or degenerate variants, thereof, that participate in the regulation, control and/or modulation of G-protein-mediated signal transduction involved in T cell activation, including, but not limited to T helper (TH) cell and TH cell subpopulation activation. Specifically, the nucleic acid mols. of the present invention include the genes corresponding to the mammalian RATH genes, including the RATH1.1 genes. Sequence anal. indicates that the RATH genes are novel genes belonging to the RGS ("regulator of G-protein signalling") gene family, a gene family which encodes gene products involved in G-protein-mediated signal transduction.

RE.CNT 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

IN ***Levinson, Douglas A.*** ; Gimeno, Carlos J.

IT ***Antibodies***

RL: BSU (Biological study, unclassified); BIOL (Biological study)
(gene RATH protein-specific; RATH genes and polypeptides and methods for the treatment and diagnosis of immune disorders)

L3 ANSWER 6 OF 13 CAPLUS COPYRIGHT 2003 ACS on STN

AN 1990:513464 CAPLUS

DN 113:113464

TI A bifunctional fusion protein containing Fc-binding fragment B of staphylococcal protein A amino-terminal to antidigoxin single-chain Fv
AU Tai, Mei Sheng; Mudgett-Hunter, Meredith; ***Levinson, Douglas*** ; Wu, Gay May; Haber, Edgar; Oppermann, Hermann; Huston, James S.

CS Creat. BioMol., Inc., Hopkinton, MA, 01748, USA

SO Biochemistry (1990), 29(35), 8024-30

CODEN: BICHAW; ISSN: 0006-2960

DT Journal

LA English

AB A bifunctional mol. was genetically engineered that which contained an amino-terminal effector domain that bound Ig Fc (fragment B of staphylococcal protein A) and a carboxyl-terminal domain that bound digoxin [a single-chain Fv (sFv)]. Effector and sFv binding properties

were virtually identical with those of the parent mols., despite the proximity of the FB to the sFv combining site. This finding is unprecedented since in all mols. of the natural Ig superfamily, the antigen binding domain is amino terminal to the effector domain. The FB-sFv sequence was encoded in a single synthetic gene and expressed as a 33,106 mol. wt. protein in *Escherichia coli*. After purifn., renaturation, and affinity isolation, yields of active fusion protein were 110 mg/L of fermented cells (18.5-g cell paste). Bifunctionality was confirmed by the ability of FB-sFv to cross-link IgG to digoxin-bovine serum albumin, as measured by plate assays and by Ouchterlony anal. Anal. of the expressed fusion protein suggests that the sFv holds promise for the development of multifunctional, targetable single-chain proteins.

AU Tai, Mei Sheng; Mudgett-Hunter, Meredith; ***Levinson, Douglas*** ; Wu, Gay May; Haber, Edgar; Oppermann, Hermann; Huston, James S.

ST Staphylococcus protein A fusion product; digoxin ***antibody*** fusion product protein A

IT 20830-75-5, Digoxin

RL: BIOL (Biological study)

(***antibody*** to, single-chain Fv based on, fusion product with staphylococcal protein A Fc-binding fragment, genetic engineering of)

L3 ANSWER 7 OF 13 CAPLUS COPYRIGHT 2003 ACS on STN

AN 1989:455415 CAPLUS

DN 111:55415

TI Protein engineering of ***antibody*** binding sites: recovery of specific activity in an anti-digoxin single-chain Fv analog produced in *Escherichia coli*

AU Huston, James S.; ***Levinson, Douglas*** ; Mudgett-Hunter, Meredith; Tai, Mei Sheng; Novotny, Jiri; Margolies, Michael N.; Ridge, Richard J.; Brucoleri, Robert E.; Haber, Edgar; et al.

CS Creat. Biomol., Hopkinton, MA, 01748, USA

SO Proceedings of the National Academy of Sciences of the United States of America (1988), 85(16), 5879-83

CODEN: PNASA6; ISSN: 0027-8424

DT Journal

LA English

AB A biosynthetic ***antibody*** binding site, which incorporated the variable domains of anti-digoxin monoclonal ***antibody*** 26-10 in a single polypeptide chain ($M_r = 26,354$), was produced in *E. coli* by protein engineering. This variable region fragment (Fv) analog comprised the 26-10 heavy- and light-chain variable regions (VH and VL) connected by a 15-amino acid linker to form a single-chain Fv (sFv). The sFv was designed as a prolyl-VH-(linker)-VL sequence of 248 amino acids. A 744-base-pair DNA sequence corresponding to this sFv protein was derived by using an *E. coli* codon preference, and the sFv gene was assembled starting from synthetic oligonucleotides. The sFv polypeptide was expressed as a fusion protein in *E. coli*, using a leader derived from the trp LE sequence. The sFv protein was obtained by acid cleavage of the unique Asp-Pro peptide bond engineered at the junction of leader and sFv in the fusion protein [(leader)-Asp-Pro-VH-(linker)-VL]. After isolation and renaturation, folded sFv displayed specificity for digoxin and related cardiac glycosides similar to that of natural 26-10 Fab fragments. Binding between affinity-purified sFv and digoxin exhibited an assocn. const. ($K_a = 3.2 \cdot 10^7 M^{-1}$) that was about a factor of 6 smaller than that found for 26-10 Fab fragments ($K_a = 1.9 \cdot 10^8 M^{-1}$) under the

same buffer conditions, consisting of 0.01M sodium acetate, pH 5.5/0.25M urea.

TI Protein engineering of ***antibody*** binding sites: recovery of specific activity in an anti-digoxin single-chain Fv analog produced in Escherichia coli

AU Huston, James S.; ***Levinson, Douglas*** ; Mudgett-Hunter, Meredith; Tai, Mei Sheng; Novotny, Jiri; Margolies, Michael N.; Ridge, Richard J.; Brucoleri, Robert E.; Haber, Edgar; et al.

AB A biosynthetic ***antibody*** binding site, which incorporated the variable domains of anti-digoxin monoclonal ***antibody*** 26-10 in a single polypeptide chain ($M_r = 26,354$), was produced in E. coli by protein engineering. This variable region fragment (Fv) analog comprised the 26-10 heavy- and light-chain variable regions (VH and VL) connected by a 15-amino acid linker to form a single-chain Fv (sFv). The sFv was designed as a prolyl-VH-(linker)-VL sequence of 248 amino acids. A 744-base-pair DNA sequence corresponding to this sFv protein was derived by using an E. coli codon preference, and the sFv gene was assembled starting from synthetic oligonucleotides. The sFv polypeptide was expressed as a fusion protein in E. coli, using a leader derived from the trp LE sequence. The sFv protein was obtained by acid cleavage of the unique Asp-Pro peptide bond engineered at the junction of leader and sFv in the fusion protein [(leader)-Asp-Pro-VH-(linker)-VL]. After isolation and renaturation, folded sFv displayed specificity for digoxin and related cardiac glycosides similar to that of natural 26-10 Fab fragments. Binding between affinity-purified sFv and digoxin exhibited an assocn. const. ($K_a = 3.2 \cdot 10^7 M^{-1}$) that was about a factor of 6 smaller than that found for 26-10 Fab fragments ($K_a = 1.9 \cdot 10^8 M^{-1}$) under the same buffer conditions, consisting of 0.01M sodium acetate, pH 5.5/0.25M urea.

L3 ANSWER 8 OF 13 USPATFULL on STN

AN 2003:226586 USPATFULL

TI Compositions and methods for the treatment and diagnosis of immune disorders

IN ***Levinson, Douglas Adam*** , Sherborn, MA, UNITED STATES

PA Millennium Pharmaceuticals, Inc. (U.S. corporation)

PI US 2003158399 A1 20030821

AI US 2002-252131 A1 20020920 (10)

RLI Continuation of Ser. No. US 1999-464231, filed on 15 Dec 1999, PENDING

Division of Ser. No. US 1996-609583, filed on 1 Mar 1996, GRANTED, Pat.

No. US 6204371 Continuation-in-part of Ser. No. US 1995-487748, filed on

7 Jun 1995, GRANTED, Pat. No. US 5721351 Continuation-in-part of Ser.

No. US 1995-398633, filed on 3 Mar 1995, GRANTED, Pat. No. US 6066322

DT Utility

FS APPLICATION

LREP PENNIE AND EDMONDS, 1155 AVENUE OF THE AMERICAS, NEW YORK, NY, 100362711

CLMN Number of Claims: 74

ECL Exemplary Claim: 1

DRWN 29 Drawing Page(s)

LN.CNT 6802

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to methods and compositions for the treatment and diagnosis of immune disorders, especially T helper lymphocyte-related disorders. For example, genes which are differentially expressed within and among T helper (TH) cells and TH

cell subpopulations, which include, but are not limited to TH0, TH1 and TH2 cell subpopulations are identified. Genes are also identified via the ability of their gene products to interact with gene products involved in the differentiation, maintenance and effector function of such TH cells and TH cell subpopulations. The genes identified can be used diagnostically or as targets for therapeutic intervention. In this regard, the present invention provides methods for the identification and therapeutic use of compounds as treatments of immune disorders, especially TH cell subpopulation-related disorders. Additionally, methods are provided for the diagnostic evaluation and prognosis of TH cell subpopulation-related disorders, for the identification of subjects exhibiting a predisposition to such conditions, for monitoring patients undergoing clinical evaluation for the treatment of such disorders, and for monitoring the efficacy of compounds used in clinical trials.

IN ***Levinson, Douglas Adam***, Sherborn, MA, UNITED STATES

SUMM . . . TH cells are involved in both humoral and cell-mediated forms of effector immune responses. With respect to the humoral, or ***antibody***, immune response, ***antibodies*** are produced by B lymphocytes through interactions with TH cells. Specifically, extracellular antigens are endocytosed by antigen-presenting cells (APCs), processed, . . . complex (MHC) molecules to CD4.sup.+ class II MHC-restricted TH cells. These TH cells in turn activate B lymphocytes, resulting in ***antibody*** production.

SUMM . . . cells drive humoral immune responses, which are associated with, for example, defenses against certain helminthic parasites, and are involved in ***antibody*** and allergic responses.

SUMM [0025] The present invention also includes the products of such fingerprint, target, and pathway genes, as well as ***antibodies*** to such gene products. Furthermore, the engineering and use of cell- and animal-based models of TH cell subpopulation-related disorders to. . .

SUMM . . . of 10 gene product activity, can be particularly amenable to modulation. For example, natural ligands, derivatives of natural ligands and ***antibodies*** which bind to the 10 gene product can be utilized to reduce the number of induced T cells present by. . .

SUMM . . . product, including, for example, soluble fusion proteins such as Ig-tailed fusion proteins, can be particularly useful for this purpose. Additionally, ***antibodies*** directed against one or more of the extracellular portions of the 10 gene product may either reduce 10 gene product function by, for example, blocking ligand binding. Additionally, ***antibodies*** directed against the 10 gene product can, in certain instances, serve to increase the level of 10 gene product activity.

SUMM . . . IgE immune response, including but not limited to the symptoms which accompany atopic conditions such as allergy and/or asthma.

IgE-type ***antibodies*** are produced by stimulated B cells which require, at least in part, IL-4 produced by the TH2 cell subpopulation. Therefore,. . .

SUMM . . . one of its gene products is a membrane-bound TH2 cell subpopulation molecule. Accordingly, natural ligands, derivatives of natural ligands and ***antibodies*** which bind to this 103 gene product, can be utilized to reduce the number of TH2 cells present by either. . .

SUMM . . . cell subpopulation-restricted, but the Ig superfamily 200 gene product is, additionally, membrane-bound. Therefore, natural ligands, derivatives of natural ligands and ***antibodies*** which bind to

the 200 gene product can be utilized to reduce the number of TH1 cells present by either. . .

SUMM . . . D10.G4, DAX; TH1: AE7.A, Dorris, D1.1). Clones were either unstimulated (-) or stimulated (+) for 6 hours with plate-bound anti-CD3 ***antibody***. Ten micrograms of total RNA were loaded per lane. The positions of 18s and 28s ribosomal RNA are shown as. . .

SUMM . . . 6, brain; lane 7, heart; lane 8, lung; lane 9, spleen; lane 10, liver. Clones were stimulated with plate-bound anti-CD3 ***antibody*** for 6 hours. 7.5 and 10 micrograms total RNA was used for each cell line and each tissue, respectively. a,. . .

SUMM . . . D10.G4, DAX; TH1: AE7.A, Dorris, D1.1). Clones were either unstimulated (-) or stimulated (+) for 6 hours with plate-bound anti-CD3 ***antibody***. The positions of RNA markers, in kilobases, are shown for reference. The arrow marks the position of 200 gene mRNA.

SUMM . . . (D1.1, Dorris, AE7) cell lines and TH2 (D10.G4, DAX, CDC25) cell lines, either stimulated (+) or unstimulated (-) with anti-CD3 ***antibodies***. 15 micrograms of total RNA were loaded per lane.

Cells were stimulated between 6 and 7 hours with anti-CD3

antibodies, as described, below, in Section 8.1. The Northern blots were hybridized with a probe made from the entire band 54. . .

SUMM . . . (DAX, CDC25) was isolated from either unstimulated cells or from cells which had been stimulated for two hours with anti-CD3

antibodies. 15 micrograms total RNA were loaded per lane. A band 54 DNA probe was used for hybridization.

DETD [0091] Further, the gene products of such fingerprint, target, and pathway genes are described in Section 5.5, ***antibodies*** to such gene products are described in Section 5.6, as are cell- and animal-based models of TH cell subpopulation differentiation. . .

DETD . . . limited to, pharmacological methods, such as exposure to phorbol esters, calcium ionophores, or lectins (e.g., Concanavalin A), by treatment with ***antibodies*** directed against T-cell receptor epitopes (e.g., anti-CD3 ***antibodies***) or exposure, in the presence of an appropriate antigen presenting cell (APC), to an antigen that the particular TH cells. . .

DETD . . . maintenance, and/or effector function of the subpopulations, using this protein in a manner similar to the well known technique of ***antibody*** probing of .lambda.gt11 libraries.

DETD . . . mutant allele. In this manner, gene products made by the putatively mutant tissue can be expressed and screened using standard ***antibody*** screening techniques in conjunction with ***antibodies*** raised against the normal gene product, as described, below, in Section 5.6. (For screening techniques, see, for example, Harlow, E. and Lane, eds., 1988, " ***Antibodies*** : A Laboratory Manual", Cold Spring Harbor Press, Cold Spring Harbor.) In cases where the mutation results in an expressed gene product with altered function (e.g., as a result of a missense mutation), a polyclonal set of ***antibodies*** are likely to cross-react with the mutant gene product. Library clones detected via their reaction with such labeled ***antibodies*** can be purified and subjected to sequence analysis as described in this Section, above. .

DETD . . . being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of ***antibodies*** or to screen peptide libraries, for example, vectors which direct the expression of high levels of fusion protein products that. . .

DETD [0186] Alternatively, any fusion protein may be readily purified by

utilizing an ***antibody*** specific for the fusion protein being expressed. For example, a system described by Janknecht et al. allows for the ready. . .

DETD [0188] Indirect labeling involves the use of a protein, such as a labeled ***antibody***, which specifically binds to either a differentially expressed or pathway gene product. Such ***antibodies*** include but are not limited to polyclonal, monoclonal, chimeric, single chain, Fab fragments and fragments produced by an Fab expression. . .

DETD 5.6. ***Antibodies*** Specific for Differentially Expressed or Pathway Gene Products

DETD [0195] Described herein are methods for the production of ***antibodies*** capable of specifically recognizing one or more differentially expressed or pathway gene product epitopes. Such ***antibodies*** can include, but are not limited to, polyclonal ***antibodies***, monoclonal ***antibodies*** (mAbs), humanized or chimeric ***antibodies***, single chain ***antibodies***, Fab fragments, F(ab').sub.2 fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) ***antibodies***, and epitope-binding fragments of any of the above. The Ig tails of such ***antibodies*** can be modified to reduce complement activation and Fc binding. (See, for example, European Patent No. 239400 B1, Aug. 3, . .

DETD [0196] Such ***antibodies*** can be used, for example, in the detection of a fingerprint, target, or pathway gene product in a biological sample, and can be used as part of diagnostic techniques. Alternatively, such ***antibodies*** can be utilized as part of an immune disorder treatment method, as described, below, in Section 5.9. For example, the ***antibodies*** can be used to modulate target gene activity, can be used to modulate TH cell subpopulation differentiation, maintenance and/or effector function, or, in the case of ***antibodies*** directed to cell surface epitopes, can be used to isolate a TH cell subpopulation of interest, for either depletion or. . .

DETD [0197] For the production of ***antibodies*** to a differentially expressed or pathway gene, various host animals can be immunized by injection with a differentially expressed or. . .

DETD [0198] Polyclonal ***antibodies*** are heterogeneous populations of ***antibody*** molecules derived from the sera of animals immunized with an antigen, such as target gene product, or an antigenic functional derivative thereof. For the production of polyclonal ***antibodies***, host animals such as those described above, can be immunized by injection with differentially expressed or pathway gene product supplemented. . .

DETD [0199] Monoclonal ***antibodies***, which are homogeneous populations of ***antibodies*** to a particular antigen, can be obtained by any technique which provides for the production of ***antibody*** molecules by continuous cell lines in culture. These include, but are not limited to the hybridoma technique of Kohler and. . . 4:72; Cole et al., 1983, Proc. Natl. Acad. Sci. USA 80:2026-2030), and the EBV-hybridoma technique (Cole et al., 1985, Monoclonal ***Antibodies*** And Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). Such ***antibodies*** can be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD and any subclass thereof. The hybridoma producing the. . .

DETD [0200] In addition, techniques developed for the production of "chimeric ***antibodies***" (Morrison et al., 1984, Proc. Natl. Acad. Sci., 81:6851-6855; Neuberger et al., 1984, Nature, 312:604-608; Takeda et al., 1985, Nature, 314:452-454; U.S. Pat. No. 4,816,567) by splicing the genes from a mouse ***antibody*** molecule of appropriate antigen specificity together with genes from a human ***antibody*** molecule of appropriate biological activity can be used. A chimeric ***antibody*** is a molecule in which different portions are derived from different animal species, such as those having a variable region.

DETD [0201] Alternatively, techniques described for the production of single chain ***antibodies*** (U.S. Pat. No. 4,946,778; Bird, 1988, Science 242:423-426; Huston et al., 1988, Proc. Natl. Acad. Sci. USA 85:5879-5883; and Ward et al., 1989, Nature 334:544-546) and for making humanized monoclonal ***antibodies*** (U.S. Pat. No. 5,225,539, which is incorporated herein by reference in its entirety) can be utilized to produce anti-differentially expressed or anti-pathway gene product ***antibodies***.

DETD [0202] ***Antibody*** fragments which recognize specific epitopes can be generated by known techniques. For example, such fragments include but are not limited to: the F(ab').sub.2 fragments which can be produced by pepsin digestion of the ***antibody*** molecule and the Fab fragments which can be generated by reducing the disulfide bridges of the F(ab').sub.2 fragments. Alternatively, Fab.

DETD [0203] ***Antibodies*** to the differentially expressed or pathway gene products can, in turn, be utilized to generate anti-idiotypic ***antibodies*** that "mimic" such gene products, using techniques well known to those skilled in the art. (See, e., Greenspan & Bona, . . Nissinoff, 1991, J. Immunol. 147(8):2429-2438). For example, in the case of receptor-type molecules (e.g., 10, 103 and 200 gene products) ***antibodies*** which bind to the ECD and competitively inhibit the binding of ligand to the receptor can be used to generate.

DETD . . . from the animal, in situ hybridization analysis, and RT-PCR. Samples of target gene-expressing tissue, can also be evaluated immunocytochemically using ***antibodies*** specific for the target gene transgene gene product of interest.

DETD [0216] The target gene transgenic animals that express target gene mRNA or target gene transgene peptide (detected immunocytochemically, using ***antibodies*** directed against target gene product epitopes) at easily detectable levels can then be further evaluated to identify those animals which.

DETD . . . limited to members of random or partially degenerate, directed phosphopeptide libraries; see, e.g., Songyang, Z. et al., 1993, Cell 72:767-778), ***antibodies*** (including, but not limited to polyclonal, monoclonal, humanized, anti-idiotypic, chimeric or single chain ***antibodies***, and FAb, F(ab').sub.2 and FAb expression library fragments, and epitope-binding fragments thereof), and small organic or inorganic molecules. In the. . . bind to the ECD and either mimic the activity triggered by the natural ligand (i.e., agonists); as well as peptides, ***antibodies*** or fragments thereof, and other organic compounds that mimic the ECD (or a portion thereof) and bind to a "neutralize". . .

DETD . . . can be accomplished by simply coating the solid surface with a solution of the protein and drying. Alternatively, an immobilized ***antibody***, preferably a monoclonal ***antibody***, specific

for the protein to be immobilized can be used to anchor the protein to the solid surface. The surfaces. . .

DETD . . . is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; e.g., using a labeled ***antibody*** specific for the previously nonimmobilized component (the ***antibody***, in turn, can be directly labeled or indirectly labeled with a labeled anti-Ig ***antibody***).

DETD . . . be conducted in a liquid phase, the reaction products separated from unreacted components, and complexes detected; e.g., using an immobilized ***antibody*** specific for target gene product or the test compound to anchor any complexes formed in solution, and a labeled ***antibody*** specific for the other component of the possible complex to detect anchored complexes.

DETD . . . target gene protein, especially mutant target gene proteins. Such compounds can include, but are not limited to molecules such as ***antibodies***, peptides, and the like, as described, for example, in Section 5.8.1. above.

DETD . . . coating the solid surface with a solution of the target gene product or binding partner and drying. Alternatively, an immobilized ***antibody*** specific for the species to be anchored can be used to anchor the species to the solid surface. The surfaces. . .

DETD . . . is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; e.g., using a labeled ***antibody*** specific for the initially non-immobilized species (the ***antibody***, in turn, can be directly labeled or indirectly labeled with a labeled anti-Ig ***antibody***). Depending upon the order of addition of reaction components, test compounds which inhibit complex formation or which disrupt preformed complexes. . .

DETD . . . or absence of the test compound, the reaction products separated from unreacted components, and complexes detected; e.g., using an immobilized ***antibody*** specific for one of the binding components to anchor any complexes formed in solution, and a labeled ***antibody*** specific for the other partner to detect anchored complexes. Again, depending upon the order of addition of reactants to the. . .

DETD . . . the resulting fusion protein. The interactive cellular or extracellular binding partner can be purified and used to raise a monoclonal ***antibody***, using methods routinely practiced in the art and described above, in Section 5.6. This ***antibody*** can be labeled with the radioactive isotope ¹²⁵I, for example, by methods routinely practiced in the art. In a heterogeneous. . . binding to occur. At the end of the reaction period, unbound material can be washed away, and the labeled monoclonal ***antibody*** can be added to the system and allowed to bind to the complexed components. The interaction between the target gene. . .

DETD . . . away. Again the extent of inhibition of the target gene product/binding partner interaction can be detected by adding the labeled ***antibody*** and measuring the radioactivity associated with the beads.

DETD . . . limited to members of random or partially degenerate, directed phosphopeptide libraries; see, e.g., Songyang, Z. et al., 1993, Cell 72:767-778), ***antibodies*** (including, but not limited to polyclonal, monoclonal, humanized, anti-idiotypic, chimeric or single chain ***antibodies***, and FAb, F(ab').sub.2 and FAb expression library fragments, and epitope-binding fragments thereof, and small

organic or inorganic molecules.

DETD . . . soluble Ig-tailed fusion proteins which may be produced by utilizing techniques such as those described, above, in Section 5.5. Additionally, ***antibodies*** directed against the extracellular portion of the 10 gene product may reduce 10 gene product function by, for example, blocking. . .

DETD . . . for example, peptides representing soluble extracellular portions of target gene product transmembrane receptors), phosphopeptides, small organic or inorganic molecules, or ***antibodies*** (including, for example, polyclonal, monoclonal, humanized, anti-idiotypic, chimeric or single chain ***antibodies***, and FAb, F(ab').sub.2 and FAb expression library fragments, and epitope-binding fragments thereof). Techniques for the determination of effective doses and. . .

DETD . . . IgE immune responses, including, but not limited to the symptoms which accompany atopic conditions such as allergy and/or asthma. IgE-type ***antibodies*** are produced by stimulated B cells which require, at least in part, IL-4 produced by the TH2 cell subpopulation. Therefore,. . .

DETD . . . a reduction in the activity and/or effective concentration of TH2 cells. For example, natural ligands, derivatives of natural ligands and ***antibodies*** which bind to the 103 gene product can be utilized to reduce the number of TH2 cells present by either. . .

DETD . . . a reduction in the activity and/or effective concentration of TH1 cells. For example, natural ligands, derivatives of natural ligands and ***antibodies*** which bind to the 200 gene product can be utilized to reduce the number of TH1 cells present by either. . .

DETD . . . and/or analogs thereof, of the gene 200 product, including, for example, soluble fusion proteins such as Ig-tailed fusion proteins or ***antibodies***. (For a discussion of the production of Ig-tailed fusion proteins see, for example, U.S. Pat. No. 5, 116,964.)

DETD . . . these 200 gene product domains or mutants fused to another polypeptide (e.g., an IgFc polypeptide) can be utilized. Alternatively, anti-idiotypic ***antibodies*** or Fab fragments of antiidiotypic ***antibodies*** that mimic the 200 gene product ECD and neutralize 200 gene product ligand can be used. Such 200 gene product peptides, proteins, fusion proteins, anti-idiotypic ***antibodies*** or Fabs are administered to a subject in amounts sufficient to neutralize Ob and to effectuate an amelioration of a. . .

DETD . . . into the tissue site, or modified antisense molecules, designed to target the desired cells (e.g., antisense linked to peptides or ***antibodies*** that specifically bind receptors or antigens expressed on the target cell surface) can be administered systemically.

DETD . . . limited to peptides representing soluble extracellular portions of target gene product transmembrane proteins, phosphopeptides, small organic or inorganic molecules, or ***antibodies*** (including, for example, polyclonal, monoclonal, humanized, anti-idiotypic, chimeric or single chain ***antibodies***, and FAb, F(ab').sub.2 and FAb expression library fragments, and epitope-binding fragments thereof).

DETD 5.9.3.1. ***Antibody*** Techniques

DETD [0337] ***Antibodies*** exhibiting modulatory capability can be utilized to ameliorate immune disorders such as TH cell subpopulation-related disorders. Depending on the specific ***antibody***, the modulatory effect can be negative and can, therefore, be utilized as part of the techniques described, above, in

Section. . .

DETD [0338] An ***antibody*** having negative modulatory capability refers to an ***antibody*** which specifically binds to and interferes with the action of a protein. In the case of an extracellular receptor, for example, such an ***antibody*** would specifically bind the extracellular domain of the receptor in a manner which does not activate the receptor but which disrupts the ability of the receptor to bind its natural ligand. For example, ***antibodies*** directed against the extracellular domains of genes 103 or 200 can function as such negative modulators. Additionally, ***antibodies*** directed against one or more of the 10 gene product extracellular domains can function in a negative modulatory manner. Such ***antibodies*** can be generated using standard techniques described in Section 5.6, above, against full length wild type or mutant proteins, or against peptides corresponding to portions of the proteins. The ***antibodies*** include but are not limited to polyclonal, monoclonal, FAb fragments, single chain ***antibodies***, chimeric ***antibodies***, and the like.

DETD [0339] An ***antibody*** having positive modulatory capability refers to an ***antibody*** which specifically binds to a protein and, by binding, serves to, either directly or indirectly, activate the function of the protein which it recognizes. For example, an ***antibody*** can bind to the extracellular portion of a transmembrane protein in a manner which causes the transmembrane protein to function as though its endogenous ligand was binding, thus activating, for example, a signal transduction pathway. ***antibodies*** can be generated using standard techniques described in Section 5.6, above, against full length wild type or mutant proteins, or against peptides corresponding to portions of the proteins. The ***antibodies*** include but are not limited to polyclonal, monoclonal, FAb fragments, single chain ***antibodies***, chimeric ***antibodies***, and the like.

DETD [0340] In instances where the protein, such as a target gene protein, to which the ***antibody*** is directed is intracellular and whole ***antibodies*** are used, internalizing ***antibodies*** can be preferred. However, lipofectin or liposomes can be used to deliver the ***antibody*** or a fragment of the Fab region which binds to the gene product epitope into cells. Where fragments of the ***antibody*** are used, the smallest inhibitory fragment which binds to the protein's binding domain is preferred. For example, peptides having an amino acid sequence corresponding to the domain of the variable region of the ***antibody*** that binds to the protein can be used. Such peptides can be synthesized chemically or produced via recombinant DNA technology. . . methods well known in the art (e.g., see Creighton, 1983, supra; and Sambrook et al., 1989, above). Alternatively, single chain ***antibodies***, such as neutralizing ***antibodies***, which bind to intracellular epitopes can also be administered. Such single chain ***antibodies*** can be administered, for example, by expressing nucleotide sequences encoding single-chain ***antibodies*** within the target cell population by utilizing, for example, techniques such as those described in Marasco et al. (Marasco, W. . .

DETD [0341] In instances where the protein to which the ***antibody*** is directed is extracellular, or is a transmembrane protein, any of the administration techniques described, below in Section 5.10 which are appropriate for peptide administration can be utilized to effectively

administer the ***antibodies*** to their site of action.

DETD . . . of the separation is to increase or augment the number of cells belonging to a specific TH cell subpopulation, the ***antibodies*** used can also be specific to surface markers present on undifferentiated or partially undifferentiated TH cells. After separation, and purification. . .

DETD . . . such as TH1 or TH2 cells, can be removed, and thus selectively separated and purified, by various methods which utilize ***antibodies*** which bind specific markers present on the T cell population of interest, while absent on other cells within the starting. . . a fluorescence activated cell sorter (FACS) and specific fluorochromes, biotin-avidin or biotin-streptavidin separations using biotin conjugated to cell surface marker-specific ***antibodies*** and avidin or streptavidin bound to a solid support such as affinity column matrix or plastic surfaces or magnetic separations using ***antibody***-coated magnetic beads.

DETD [0348] Separation via ***antibodies*** for specific markers can be by negative or positive selection procedures. In negative separation, ***antibodies*** are used which are specific for markers present on undesired cells. For example, in the case of a TH1 cell subpopulation-related disorder wherein it would be desirable to deplete the number of TH1 cells, such ***antibodies*** could be directed to the extracellular domain of the 200 gene product. Alternatively, in the case of TH2 cell subpopulation-related disorders wherein it would be desirable to deplete the number of TH1 cells, such ***antibodies*** could be directed to the extracellular domain of the 103 gene product. Cells bound by an ***antibody*** to such a cell surface marker can be removed or lysed and the remaining desired mixture retained.

DETD [0349] In positive separation, ***antibodies*** specific for markers present on the desired cells of interest. For example, in the case of a TH1 cell subpopulation-related disorder wherein it would be desirable to increase the number of TH1 cells, such ***antibodies*** could be directed to the extracellular domain of the 200 gene product. Alternatively, in the case of TH2 cell subpopulation-related disorders wherein it would be desirable to increase the number of TH1 cells, such ***antibodies*** could be directed to the extracellular domain of the 103 gene product. Cells bound by the ***antibody*** are separated and retained. It will be understood that positive and negative separations can be used substantially simultaneously or in. . .

DETD [0350] A common technique for ***antibody*** based separation is the use of flow cytometry such as by a fluorescence activated cell sorter (FACS). Typically, separation by flow cytometry is performed as follows. The suspended mixture of cells are centrifuged and resuspended in media. ***Antibodies*** which are conjugated to fluorochrome are added to allow the binding of the ***antibodies*** to specific cell surface markers. The cell mixture is then washed by one or more centrifugation and resuspension steps. The. . .

DETD . . . such method is biotin-avidin based separation by affinity chromatography. Typically, such a technique is performed by incubating cells with biotin-coupled ***antibodies*** to specific markers, such as, for example, the transmembrane protein encoded by the 103 gene described herein, followed by passage through an avidin column. Biotin-***antibody***-cell complexes bind to the column via the biotin-avidin interaction, while other cells pass through the column. The specificity of the. . .

DETD . . . proliferation of the purified TH cell subpopulation. Such an expansion agent can, for example, be any appropriate cytokine, antigen, or ***antibody***. In the case of TH2 cells, for example, the expansion agent can be IL-4, while for TH1 cells, the expansion. . .

DETD [0360] Targeting moieties can include, but are not limited to, ***antibodies*** directed to cell surface markers found specifically on the TH cell subpopulation being targeted, or, alternatively, to ligands, such as. . .

DETD [0361] In the case of TH2 cells, for example, such a targeting moiety can represent an ***antibody*** directed against the extracellular portion of the 103 gene product described herein, or can, alternatively, represent a ligand specific for. . . this receptor-type TH2 specific molecule. In the case of TH1 cells, for example, such a targeting moiety can represent an ***antibody*** directed against the extracellular portion of the 200 gene product described herein, or can, alternatively, represent a ligand specific for. . .

DETD [0377] Such methods can, for example, utilize reagents such as the fingerprint gene nucleotide sequences described in Sections 5.1, and ***antibodies*** directed against differentially expressed and pathway gene peptides, as described, above, in Sections 5.5 (peptides) and 5.6 (***antibodies***). Specifically, such reagents can be used, for example, for: 1) the detection of the presence of target gene expression, target. . .

DETD . . . performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one specific fingerprint gene nucleic acid or anti-fingerprint gene ***antibody*** reagent described herein, which can be conveniently used, e.g., in clinical settings, to diagnose patients exhibiting TH1- or TH2-related abnormalities.

DETD [0398] ***Antibodies*** directed against wild type or mutant fingerprint gene peptides, which are discussed, above, in Section 5.6, can also be used. . .

DETD . . . employed herein can, for example, be such as those described in Harlow and Lane (Harlow, E. and Lane, D., 1988, " ***Antibodies*** : A Laboratory Manual", Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.), which is incorporated herein by reference in its. . .

DETD . . . molecules can involve, for example, immunoassays wherein fingerprint gene peptides are detected by their interaction with an anti-fingerprint gene product-specific ***antibody***.

DETD [0401] For example, ***antibodies***, or fragments of ***antibodies***, such as those described, above, in Section 5.6, useful in the present invention can be used to quantitatively or qualitatively. . . wild type or mutant fingerprint gene peptides. This can be accomplished, for example, by immunofluorescence techniques employing a fluorescently labeled ***antibody*** (see below, this Section,) coupled with light microscopic, flow cytometric, or fluorimetric detection. Such techniques are especially preferred if the.

DETD [0402] The ***antibodies*** (or fragments thereof) useful in the present invention can, additionally, be employed histologically, as in immunofluorescence or immunoelectron microscopy, for. . . peptides. In situ detection can be accomplished by removing a histological specimen from a patient, and applying thereto a labeled ***antibody*** of the present invention. The ***antibody*** (or fragment) is preferably applied by overlaying the labeled ***antibody*** (or fragment) onto a biological sample. Through the use of such a procedure,

it is possible to determine not only. . .

DETD . . . extract, freshly harvested cells, or cells which have been incubated in tissue culture, in the presence of a detectably labeled ***antibody*** capable of identifying fingerprint gene peptides, and detecting the bound ***antibody*** by any of a number of techniques well-known in the art.

DETD . . . soluble proteins. The support can then be washed with suitable buffers followed by treatment with the detectably labeled fingerprint gene-specific ***antibody***. The solid phase support can then be washed with the buffer a second time to remove unbound ***antibody***. The amount of bound label on solid support can then be detected by conventional means.

DETD [0405] By "solid phase support or carrier" is intended any support capable of binding an antigen or an ***antibody***. Well-known supports or carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, gabbros, and magnetite. . . have virtually any possible structural configuration so long as the coupled molecule is capable of binding to an antigen or ***antibody***. Thus, the support configuration can be spherical, as in a bead, or cylindrical, as in the inside surface of a . . . strip, etc. Preferred supports include polystyrene beads. Those skilled in the art will know many other suitable carriers for binding ***antibody*** or antigen, or will be able to ascertain the same by use of routine experimentation.

DETD [0406] The binding activity of a given lot of anti-wild type or mutant fingerprint gene product ***antibody*** can be determined according to well known methods. Those skilled in the art will be able to determine operative and. . .

DETD [0407] One of the ways in which the fingerprint gene peptide-specific ***antibody*** can be detectably labeled is by linking the same to an enzyme and use in an enzyme immunoassay (EIA) (Voller, . . . Raton, Fla.; Ishikawa, E. et al., (eds.), 1981, ENZYME IMMUNOASSAY, Kogaku Shoin, Tokyo). The enzyme which is bound to the ***antibody*** will react with an appropriate substrate, preferably a chromogenic substrate, in such a manner as to produce a chemical moiety. . . be detected, for example, by spectrophotometric, fluorimetric or by visual means. Enzymes which can be used to detectably label the ***antibody*** include, but are not limited to, malate dehydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, yeast alcohol dehydrogenase, alpha-glycerophosphate, dehydrogenase, triose phosphate isomerase, . .

DETD [0408] Detection can also be accomplished using any of a variety of other immunoassays. For example, by radioactively labeling the ***antibodies*** or ***antibody*** fragments, it is possible to detect fingerprint gene wild type or mutant peptides through the use of a radioimmunoassay (RIA). . .

DETD [0409] It is also possible to label the ***antibody*** with a fluorescent compound. When the fluorescently labeled ***antibody*** is exposed to light of the proper wavelength, its presence can then be detected due to fluorescence. Among the most. . .

DETD [0410] The ***antibody*** can also be detectably labeled using fluorescence emitting metals such as .sup.152Eu, or others of the lanthanide series. These metals can be attached to the ***antibody*** using such metal chelating groups as diethylenetriaminepentaacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA).

DETD [0411] The ***antibody*** also can be detectably labeled by coupling it to a chemiluminescent compound. The presence of the chemiluminescent-tagged ***antibody*** is then determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of. . .

DETD [0412] Likewise, a bioluminescent compound can be used to label the ***antibody*** of the present invention. Bioluminescence is a type of chemiluminescence found in biological systems in, which a catalytic protein increases. . .

DETD . . . samples were isolated from either unstimulated cells or from cells which had been stimulated for 6 hours with plate-bound anti-CD3 ***antibody***. The samples were probed with band 103 sequences, as shown in FIG. 6. While 103 gene RNA is present in. . .

DETD . . . E. et al., John Wiley & Sons, NY, pp 3.12.4-3.12.6). Specifically, prior to plating, the flasks were coated with anti-CD3-.epsilon. ***antibodies*** (hybridoma supernatant from the 145-C11 hybridoma; Parmingen, Inc., San Diego Calif.). For coating, ***antibodies*** were resuspended in PBS at 1-2 .mu.g/ml at a volume sufficient to coat the bottom of the flasks. Coating solution. . .

DETD [0459] After incubation, the ***antibody*** coating solution was removed by aspiration and cells were immediately added. Flasks were placed in a 37.degree. C. incubator for. . .

DETD . . . samples were isolated from either unstimulated cells (-) or cells which had been stimulated for 6 hours with plate-bound anti-CD3 ***antibody*** (+). The samples were probed with 200 gene sequences, and, as shown in FIG. 18, RNA from both stimulated and. . .

DETD . . . blot data depicted in FIG. 19 additionally illustrates 54 gene expression within cell clones either stimulated or unstimulated with an ***antibodies***, and demonstrates that 54 gene expression within stimulated TH1 cells.

DETD . . . Specifically, RNA was isolated from unstimulated AE7 clones as well as from AE7 clones which had been stimulated with anti-CD3 ***antibodies*** for varying lengths of time, as noted in FIG. 20. As illustrated in FIG. 20, 54 gene expression decreased slightly by. . .

CLM What is claimed is:

11. An ***antibody*** that immunospecifically binds the gene product of claim 9.

L3 ANSWER 9 OF 13 USPATFULL on STN

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TI Compositions and methods for the treatment and diagnosis of immune disorders

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DT Utility

FS APPLICATION

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CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to methods and compositions for the treatment and diagnosis of immune disorders, especially T helper lymphocyte-related disorders, and also for the treatment of mast cell-related processes and disorders, ischemic disorders and injuries, including ischemic renal disorders and injuries. For example, genes which are differentially expressed within and among T helper (TH) cells and TH cell subpopulations, which include, but are not limited to TH0, TH1 and TH2 cell subpopulations are identified. Genes are also identified via the ability of their gene products to interact with gene products involved in the differentiation, maintenance and effector function of such TH cells and TH cell subpopulations. The genes identified can be used diagnostically or as targets for therapeutic intervention. In this regard, the present invention provides methods for the identification and therapeutic use of compounds as treatments of immune disorders, especially TH cell subpopulation-related disorders. Additionally, methods are provided for the diagnostic evaluation and prognosis of TH cell subpopulation-related disorders, for the identification of subjects exhibiting a predisposition to such conditions, for monitoring patients undergoing clinical evaluation for the treatment of such disorders, and for monitoring the efficacy of compounds used in clinical trials. Methods are also provided for the treatment of symptoms associated with mast cell-related processes or disorders and ischemic disorders and injuries using the genes, gene products and ***antibodies*** of the invention.

IN ***Levinson, Douglas Adam***, Sherborn, MA, UNITED STATES

AB . . . of symptoms associated with mast cell-related processes or disorders and ischemic disorders and injuries using the genes, gene products and ***antibodies*** of the invention.

SUMM . . . TH cells are involved in both humoral and cell-mediated forms of effector immune responses. With respect to the humoral, or ***antibody***, immune response, ***antibodies*** are produced by B lymphocytes through interactions with TH cells. Specifically, extracellular antigens are endocytosed by antigen-presenting cells (APCs), processed, . . . complex (MHC) molecules to CD4.sup.+ class II MHC-restricted TH cells. These TH cells in turn activate B lymphocytes, resulting in ***antibody*** production.

SUMM . . . cells drive humoral immune responses, which are associated with, for example, defenses against certain helminthic parasites, and are involved in ***antibody*** and allergic responses.

SUMM [0026] The present invention also includes the products of such fingerprint, target, and pathway genes, as well as ***antibodies*** to such gene products. Furthermore, the engineering and use of cell- and animal-based models of TH cell subpopulation-related disorders to. . .

SUMM . . . of ischemic disorders or injuries. For example, presented herein are methods of using the 200 gene, its gene product, and ***antibodies*** thereto to treat or regulate ischemic disorders

and/or injuries. In particular, the genes or gene products of the invention may. . . to an individual so as to ameliorate the symptoms of the ischemic disorder or injury. Further, compounds, such as specific ***antibodies***, including monoclonal ***antibodies***, which bind specifically to the genes or gene products of the present invention and modulate their expression or activity, may. . .

SUMM . . . of 10 gene product activity, can be particularly amenable to modulation. For example, natural ligands, derivatives of natural ligands and ***antibodies*** which bind to the 10 gene product can be utilized to reduce the number of induced T cells present by. . .

SUMM . . . product, including, for example, soluble fusion proteins such as Ig-tailed fusion proteins, can be particularly useful for this purpose. Additionally, ***antibodies*** directed against one or ore of the extracellular portions of the 10 gene product may either reduce 10 gene product function by, for example, locking ligand binding. Additionally, ***antibodies*** directed against the 10 gene product can, in certain instances, serve to increase the level of 10 gene product activity.

SUMM . . . IgE immune response, including but not limited to the symptoms which accompany atopic conditions such as allergy and/or asthma. IgE-type ***antibodies*** are produced by stimulated B cells which require, at least in part, IL-4 produced by the TH2 cell subpopulation. Therefore,. . .

SUMM . . . one of its gene products is a membrane-bound TH2 cell subpopulation molecule. Accordingly, natural ligands, derivatives of natural ligands and ***antibodies*** which bind to this 103 gene product, can be utilized to reduce the number of TH2 cells present by either. . . for example, soluble fusion proteins such as Ig-tailed fusion proteins, can be particularly useful for this purpose. In certain instances, ***antibodies*** directed against the 103 gene product, such as directed against the extracellular domain of the 103 gene product, can be. . .

SUMM . . . cell subpopulation-restricted, but the Ig superfamily 200 gene product is, additionally, membrane-bound. Therefore, natural ligands, derivatives of natural ligands and ***antibodies*** which bind to the 200 gene product can be utilized to reduce the number of TH1 cells present by either. . .

SUMM [0055] Further, the Example presented in Section 12, below, describes successful use of ***antibodies*** directed against the 103 gene product as well as 103/Ig fusion proteins to ameliorate symptoms of asthma in an accepted. . .

SUMM . . . after injury, particularly after ischemic injury. In particular, the example presented in Section 13, below, demonstrates the successful use of ***antibodies*** which bind to the extracellular domain of the 200 gene product to inhibit repair of ischemic kidney injury. Thus, the. . .

DRWD . . . D10.G4, DAX; TH1: AE7.A, Dorris, D1.1). Clones were either unstimulated (-) or stimulated (+) for 6 hours with plate-bound anti-CD3 ***antibody***. Ten micrograms of total RNA were loaded per lane. The positions of 18s and 28s ribosomal RNA are shown as. . .

DRWD . . . 6, brain; lane 7, heart; lane 8, lung; lane 9, spleen; lane 10, liver. Clones were stimulated with plate-bound anti-CD3 ***antibody*** for 6 hours. 7.5 and 10 micrograms total RNA was used for each cell line and each tissue, respectively. a,. . .

DRWD . . . D10.G4, DAX; TH1: AE7.A, Dorris, D1.1). Clones were either

unstimulated (-) or stimulated (+) for 6 hours with plate-bound anti-CD3 ***antibody***. The positions of RNA markers, in kilobases, are shown for reference. The arrow marks the position of 200 gene mRNA.

DRWD . . . (D1.1, Dorris, AE7) cell lines and TH2 (D10.G4, DAX, CDC25) cell lines, either stimulated (+) or unstimulated (-) with anti-CD3 ***antibodies***. 15 micrograms of total RNA were loaded per lane. Cells were stimulated between 6 and 7 hours with anti-CD3 ***antibodies***, as described, below, in Section 8.1. The Northern blots were hybridized with a probe made from the entire band 54. . .

DRWD . . . (DAX, CDC25) was isolated from either unstimulated cells or from cells which had been stimulated for two hours with anti-CD3 ***antibodies***. 15 micrograms total RNA were loaded per lane. A band 54 DNA probe was used for hybridization.

DRWD . . . surface 103 gene product. The further to the right the peaks are shifted, the greater the staining intensity, and therefore ***antibody*** binding, exhibited by a cell population.

DRWD . . . 103/Ig fusion results in significant decrease in hallmark symptoms of asthma. FIG. 29A: Animals were treated with the anti-103 3E10 ***antibody*** (listed in the figure as "3E10 MAB"). As a negative control, a set of animals was treated with a non-specific rat Ig ***antibody*** preparation. FIG. 29B: Animals were treated with 103/Ig fusion protein (listed in the figure as "Ig Fus. Prot.") as a negative control, a control set of animals were treated with a non-specific human IgG ***antibody*** preparation.

DRWD . . . a section of untreated mouse kidney tissue; FIG. 31B shows a section of mouse kidney tissue treated with 200 gene ***antibody*** 24 hours prior to, and at 24 hour intervals after the induction of ischemic kidney injury.

DETD [0116] Further, the gene products of such fingerprint, target, and pathway genes are described in Section 5.5, ***antibodies*** to such gene products are described in Section 5.6, as are cell- and animal-based models of TH cell subpopulation differentiation. . .

DETD . . . limited to, pharmacological methods, such as exposure to phorbol esters, calcium ionophores, or lectins (e.g., Concanavalin A), by treatment with ***antibodies*** directed against T-cell receptor epitopes (e.g., anti-CD3 ***antibodies***) or exposure, in the presence of an appropriate antigen presenting cell (APC), to an antigen that the particular TH cells. . .

DETD . . . maintenance, and/or effector function of the subpopulations, using this protein in a manner similar to the well known technique of ***antibody*** probing of .lambda.gt11 libraries.

DETD . . . the pathway and/or differentially expressed genes of the invention also include gene products which are recognized by and bind to ***antibodies*** (polyclonal or monoclonal) directed against the differentially expressed and/or pathway gene products described above; e.g., which are encoded by the. . .

DETD . . . mutant allele. In this manner, gene products made by the putatively mutant tissue can be expressed and screened using standard ***antibody*** screening techniques in conjunction with ***antibodies*** raised against the normal gene product, as described, below, in Section 5.6. (For screening techniques, see, for example, Harlow, E. and Lane, eds., 1988, " ***Antibodies*** : A Laboratory Manual", Cold Spring Harbor Press, Cold Spring Harbor.) In cases where the mutation results in an expressed gene product with altered function (e.g., as a result of a missense mutation), a polyclonal set of

antibodies are likely to cross-react with the mutant gene product. Library clones detected via their reaction with such labeled ***antibodies*** can be purified and subjected to sequence analysis as described in this Section, above.

DETD . . . as many contiguous amino acid residues as necessary to represent an epitope fragment (that is to be recognized by an ***antibody*** directed to the differentially expressed or pathway gene product). For example, such protein fragments or peptides can comprise at least. . .

DETD . . . being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of ***antibodies*** or to screen peptide libraries, for example, vectors which direct the expression of high levels of fusion protein products that. . .

DETD [0229] Alternatively, any fusion protein may be readily purified by utilizing an ***antibody*** specific for the fusion protein being expressed. For example, a system described by Janknecht et al. allows for the ready. . .

DETD [0231] Indirect labeling involves the use of a protein, such as a labeled ***antibody***, which specifically binds to either a differentially expressed or pathway gene product. Such ***antibodies*** include but are not limited to polyclonal, monoclonal, chimeric, single chain, Fab fragments and fragments produced by an Fab expression. . .

DETD 5.6. ***Antibodies*** Specific for Differentially Expressed or Pathway Gene Products

DETD [0241] Described herein are methods for the production of ***antibodies*** capable of specifically recognizing one or more differentially expressed or pathway gene product epitopes. Such ***antibodies*** can include, but are not limited to, polyclonal ***antibodies***, monoclonal ***antibodies*** (mAbs), humanized or chimeric ***antibodies***, single chain ***antibodies***, Fab fragments, F(ab').sub.2 fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) ***antibodies***, and epitope-binding fragments of any of the above. The Ig tails of such ***antibodies*** can be modified to reduce complement activation and Fc binding. (See, for example, European Patent No. 239400 B1, Aug. 3, . . .

DETD [0242] Such ***antibodies*** can be used, for example, in the detection of a fingerprint, target, or pathway gene product. in a biological sample, and can be used as part of diagnostic techniques. Alternatively, such ***antibodies*** can be utilized as part of an immune disorder treatment method, as described, below, in Section 5.9. For example, the ***antibodies*** can be used to modulate target gene activity, can be used to modulate TH cell subpopulation differentiation, maintenance and/or effector function, or, in the case of ***antibodies*** directed to cell surface epitopes, can be used to isolate a TH cell subpopulation of interest, for either depletion or. . .

DETD [0243] Such ***antibodies*** can also be utilized as part of a method for treatment of an ischemic disorder or injury, as described in Section 5.10.3.1, below. For example, the ***antibodies*** can be used to block or inhibit activity of one or more of the gene products of the invention, thereby. . .

DETD [0244] For the production of ***antibodies*** to a differentially expressed or pathway gene, various host animals can be immunized by

injection with a differentially expressed or. . .

DETD [0245] Polyclonal ***antibodies*** are heterogeneous populations of ***antibody*** molecules derived from the sera of animals immunized with an antigen, such as target gene product, or an antigenic functional derivative thereof. For the production of polyclonal ***antibodies***, host animals such as those described above, can be immunized by injection with differentially expressed or pathway gene product supplemented. . .

DETD [0246] Monoclonal ***antibodies***, which are homogeneous populations of ***antibodies*** to a particular antigen, can be obtained by any technique which provides for the production of ***antibody*** molecules by continuous cell lines in culture. These include, but are not limited to the hybridoma technique of Kohler and. . . 4:72; Cole et al., 1983, Proc. Natl. Acad. Sci. USA 80:2026-2030), and the EBV-hybridoma technique (Cole et al., 1985, Monoclonal ***Antibodies*** And Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). Such ***antibodies*** can be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD and any subclass thereof. The hybridoma producing the. . .

DETD [0247] In addition, techniques developed for the production of "chimeric ***antibodies***" (Morrison et al., 1984, Proc. Natl. Acad. Sci., 81:6851-6855; Neuberger et al., 1984, Nature, 312:604-608; Takeda et al., 1985, Nature, 314:452-454; U.S. Pat. No. 4,816,567) by splicing the genes from a mouse ***antibody*** molecule of appropriate antigen specificity together with genes from a human ***antibody*** molecule of appropriate biological activity can be used. A chimeric ***antibody*** is a molecule in which different portions are derived from different animal species, such as those having a variable region. . .

DETD [0248] Alternatively, techniques described for the production of single chain ***antibodies*** (U.S. Pat. No. 4,946,778; Bird, 1988, Science 242:423-426; Huston et al., 1988, Proc. Natl. Acad. Sci. USA 85:5879-5883; and Ward et al., 1989, Nature 334:544-546) and for making humanized monoclonal ***antibodies*** (U.S. Pat. No. 5,225,539, which is incorporated herein by reference in its entirety) can be utilized to produce anti-differentially expressed or anti-pathway gene product ***antibodies***. . .

DETD [0249] ***Antibody*** fragments which recognize specific epitopes can be generated by known techniques. For example, such fragments include but are not limited to: the F(ab').sub.2 fragments which can be produced by pepsin digestion of the ***antibody*** molecule and the Fab fragments which can be generated by reducing the disulfide bridges of the F(ab').sub.2 fragments. Alternatively, Fab. . .

DETD [0250] ***Antibodies*** to the differentially expressed or pathway gene products can, in turn, be utilized to generate anti-idiotypic ***antibodies*** that "mimic" such gene products, using techniques well known to those skilled in the art. (See, e.g., Greenspan & Bona,. . . Nissinoff, 1991, J. Immunol. 147(8):2429-2438). For example, in the case of receptor-type molecules (e.g., 10, 103 and 200 gene products) ***antibodies*** which bind to the ECD and competitively inhibit the binding of ligand to the receptor can be used to generate. . .

DETD [0251] Production of ***antibodies*** directed against the extracellular domain of the 103 gene product are described in Section 12, below. Also, production of ***antibodies*** directed against the

extracellular domain of the 200 gene product are described in Section 13, below.

DETD . . . from the animal, in situ hybridization analysis, and RT-PCR. Samples of target gene-expressing tissue, can also be evaluated immunocytochemically using ***antibodies*** specific for the target gene transgene gene product of interest.

DETD [0265] The target gene transgenic animals that express target gene mRNA or target gene transgene peptide (detected immunocytochemically, using ***antibodies*** directed against target gene product epitopes) at easily detectable levels can then be further evaluated to identify those animals which. . .

DETD . . . limited to members of random or partially degenerate, directed phosphopeptide libraries; see, e.g., Songyang, Z. et al., 1993, Cell 72:767-778), ***antibodies*** (including, but not limited to polyclonal, monoclonal, humanized, anti-idiotypic, chimeric or single chain ***antibodies***, and FAb, F(ab').sub.2 and FAb expression library fragments, and epitope-binding fragments thereof), and small organic or inorganic molecules. In the. . . bind to the ECD and either mimic the activity triggered by the natural ligand (i.e., agonists); as well as peptides, ***antibodies*** or fragments thereof, and other organic compounds that mimic the ECD (or a portion thereof) and bind to a "neutralize". . .

DETD . . . can be accomplished by simply coating the solid surface with a solution of the protein and drying. Alternatively, an immobilized ***antibody***, preferably a monoclonal ***antibody***, specific for the protein to be immobilized can be used to anchor the protein to the solid surface. The surfaces. . .

DETD . . . is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; e.g., using a labeled ***antibody*** specific for the previously nonimmobilized component (the ***antibody***, in turn, can be directly labeled or indirectly labeled with a labeled anti-Ig ***antibody***).

DETD . . . be conducted in a liquid phase, the reaction products separated from unreacted components, and complexes detected; e.g., using an immobilized ***antibody*** specific for target gene product or the test compound to anchor any complexes formed in solution, and a labeled ***antibody*** specific for the other component of the possible complex to detect anchored complexes.

DETD . . . target gene protein, especially mutant target gene proteins. Such compounds can include, but are not limited to molecules such as ***antibodies***, peptides, and the like, as described, for example, in Section 5.8.1. above.

DETD . . . coating the solid surface with a solution of the target gene product or binding partner and drying. Alternatively, an immobilized ***antibody*** specific for the species to be anchored can be used to anchor the species to the solid surface. The surfaces. . .

DETD . . . is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; e.g., using a labeled ***antibody*** specific for the initially non-immobilized species (the ***antibody***, in turn, can be directly labeled or indirectly labeled with a labeled anti-Ig ***antibody***). Depending upon the order of addition of reaction components, test compounds which inhibit complex formation or which disrupt preformed complexes. . .

DETD . . . or absence of the test compound, the reaction products separated from unreacted components, and complexes detected; e.g., using

an immobilized ***antibody*** specific for one of the binding components to anchor any complexes formed in solution, and a labeled ***antibody*** specific for the other partner to detect anchored complexes. Again, depending upon the order of addition of reactants to the. . .

DETD . . . the resulting fusion protein. The interactive cellular or extracellular binding partner can be purified and used to raise a monoclonal ***antibody***, using methods routinely practiced in the art and described above, in Section 5.6. This ***antibody*** can be labeled with the radioactive isotope ¹²⁵I, for example, by methods routinely practiced in the art. In a heterogeneous. . . binding to occur. At the end of the reaction period, unbound material can be washed away, and the labeled monoclonal ***antibody*** can be added to the system and allowed to bind to the complexed components. The interaction between the target gene. . .

DETD . . . away. Again the extent of inhibition of the target gene product/binding partner interaction can be detected by adding the labeled ***antibody*** and measuring the radioactivity associated with the beads.

DETD . . . describes the successful utilization of a 103 gene product/Ig fusion protein, as well as the successful use of a monoclonal ***antibody*** directed against the extracellular domain of the 103 gene product to ameliorate symptoms of asthma in an accepted animal model. . .

DETD . . . limited to members of random or partially degenerate, directed phosphopeptide libraries; see, e.g., Songyang, Z. et al., 1993, Cell 72:767-778), ***antibodies*** (including, but not limited to polyclonal, monoclonal, humanized, anti-idiotypic, chimeric or single chain ***antibodies***, and FAb, F(ab').sub.2 and FAb expression library fragments, and epitope-binding fragments thereof), and small organic or inorganic molecules.

DETD . . . soluble Ig-tailed fusion proteins which may be produced by utilizing techniques such as those described, above, in Section 5.5. Additionally, ***antibodies*** directed against the extracellular portion of the 10 gene product may reduce 10 gene product function by, for example, blocking. . .

DETD . . . for example, peptides representing soluble extracellular portions of target gene product transmembrane receptors), phosphopeptides, small organic or inorganic molecules, or ***antibodies*** (including, for example, polyclonal, monoclonal, humanized, anti-idiotypic, chimeric or single chain ***antibodies***, and FAb, F(ab').sub.2 and FAb expression library fragments, and epitope-binding fragments thereof). In one embodiment, for example, ***antibodies*** directed against a 103 gene product, preferably an extracellular or extracellular portion of a 103 gene product, can be utilized.. . .

DETD . . . IgE immune responses, including, but not limited to the symptoms which accompany atopic conditions such as allergy and/or asthma. IgE-type ***antibodies*** are produced by stimulated B cells which require, at least in part, IL-4 produced by the TH2 cell subpopulation. Therefore,. . .

DETD [0351] For example, natural ligands, derivatives of natural ligands and ***antibodies*** which bind to the 103 gene product can be utilized to reduce the number of TH2 cells present by either. . .

DETD [0357] For example, natural ligands, derivatives of natural ligands and

antibodies which bind to the 200 gene product can be utilized to reduce the number of TH1 cells present by either. . .

DETD . . . and/or analogs thereof, of the gene 200 product, including, for example, soluble fusion proteins such as Ig-tailed fusion proteins or ***antibodies***. (For a discussion of the production of Ig-tailed fusion proteins see, for example, U.S. Pat. No. 5,116,964.)

DETD . . . these 200 gene product domains or mutants fused to another polypeptide (e.g., an IgFc polypeptide) can be utilized. Alternatively, anti-idiotypic ***antibodies*** or Fab fragments of antiidiotypic ***antibodies*** that mimic the 200 gene product ECD and neutralize 200 gene product ligand can be used. Such 200 gene product peptides, proteins, fusion proteins, anti-idiotypic ***antibodies*** or Fabs are administered to a subject in amounts sufficient to neutralize the gene product and thereby effectuate an amelioration. . .

DETD . . . into the tissue site, or modified antisense molecules, designed to target the desired cells (e.g., antisense linked to peptides or ***antibodies*** that specifically bind receptors or antigens expressed on the target cell surface) can be administered systemically.

DETD . . . also include peptides representing soluble extracellular portions of target gene product transmembrane proteins, phosphopeptides, small organic or inorganic molecules, or ***antibodies*** (including, for example, polyclonal, monoclonal, humanized, anti-idiotypic, chimeric or single chain ***antibodies***, and FAb, F(ab.sup.1).sub.2 and FAb expression library fragments, and epitope-binding fragments thereof).

DETD 5.10.3.1. ***Antibody*** Techniques

DETD [0402] ***Antibodies*** exhibiting modulatory capability can be utilized to ameliorate immune disorders such as TH cell subpopulation-related disorders, or to treat ischemic disorders and injuries. Depending on the specific ***antibody***, the modulatory effect can be negative and can, therefore, be utilized as part of the techniques described, above, in Section. . .

DETD [0403] An ***antibody*** having negative modulatory capability refers to an ***antibody*** which specifically binds to and interferes with the action of a protein. In the case of an extracellular receptor, for example, such an ***antibody*** would specifically bind the extracellular domain of the receptor in a manner which does not activate the receptor but which disrupts the ability of the receptor to bind its natural ligand. For example, ***antibodies*** directed against the extracellular domains of genes 103 or 200 can function as such negative modulators. Additionally, ***antibodies*** directed against one or more of the 10 gene product extracellular domains can function in a negative modulatory manner. Such ***antibodies*** can be generated using standard techniques described in Section 5.6, above, against full length wild type or mutant proteins, or against peptides corresponding to portions of the proteins. The ***antibodies*** include but are not limited to polyclonal, monoclonal, FAb fragments, single chain ***antibodies***, chimeric ***antibodies***, and the like.

DETD [0404] An ***antibody*** having positive modulatory capability refers to an ***antibody*** which specifically binds to a protein and, by binding, serves to, either directly or indirectly, activate the function of the protein which it recognizes. For example, an ***antibody*** can bind to the extracellular portion of a transmembrane protein in a manner which causes the transmembrane protein

to function as though its endogenous ligand was binding, thus activating, for example, a signal transduction pathway.

antibodies can be generated using standard techniques described in Section 5.6, above, against full length wild type or mutant proteins, or against peptides corresponding to portions of the proteins. The ***antibodies*** include but are not limited to polyclonal, monoclonal, Fab fragments, single chain ***antibodies***, chimeric ***antibodies***, and the like.

DETD [0405] In instances where the protein, such as a target gene protein, to which the ***antibody*** is directed is intracellular and whole ***antibodies*** are used, internalizing ***antibodies*** can be preferred. However, lipofectin or liposomes can be used to deliver the ***antibody*** or a fragment of the Fab region which binds to the gene product epitope into cells. Where fragments of the ***antibody*** are used, the smallest inhibitory fragment which binds to the protein's binding domain is preferred. For example, peptides having an amino acid sequence corresponding to the domain of the variable region of the ***antibody*** that binds to the protein can be used. Such peptides can be synthesized chemically or produced via recombinant DNA technology. . . methods well known in the art (e.g., see Creighton, 1983, supra; and Sambrook et al., 1989, above). Alternatively, single chain ***antibodies***, such as neutralizing ***antibodies***, which bind to intracellular epitopes can also be administered. Such single chain ***antibodies*** can be administered, for example, by expressing nucleotide sequences encoding single-chain ***antibodies*** within the target cell population by utilizing, for example, techniques such as those described in Marasco et al. (Marasco, W. . .

DETD [0406] In instances where the protein to which the ***antibody*** is directed is extracellular, or is a transmembrane protein, any of the administration techniques described, below in Section 5.11 which are appropriate for peptide administration can be utilized to effectively administer the ***antibodies*** to their site of action.

DETD . . . of the separation is to increase or augment the number of cells belonging to a specific TH cell subpopulation, the ***antibodies*** used can also be specific to surface markers present on undifferentiated or partially undifferentiated TH cells. After separation, and purification. . .

DETD . . . such as TH1 or TH2 cells, can be removed, and thus selectively separated and purified, by various methods which utilize ***antibodies*** which bind specific markers present on the T cell population of interest, while absent on other cells within the starting. . . a fluorescence activated cell sorter (FACS) and specific fluorochromes, biotin-avidin or biotin-streptavidin separations using biotin conjugated to cell surface marker-specific ***antibodies*** and avidin or streptavidin bound to a solid support such as affinity column matrix or plastic surfaces or magnetic separations using ***antibody***-coated magnetic beads.

DETD [0413] Separation via ***antibodies*** for specific markers can be by negative or positive selection procedures. In negative separation, ***antibodies*** are used which are specific for markers present on undesired cells. For example, in the case of a TH1 cell subpopulation-related disorder wherein it would be desirable to deplete the number of TH1 cells, such ***antibodies*** could be directed to the extracellular domain of the 200 gene product. Alternatively, in the case of TH2 cell subpopulation-related disorders wherein it would be

desirable to deplete the number of TH1 cells, such ***antibodies*** could be directed to the extracellular domain of the 103 gene product. Cells bound by an ***antibody*** to such a cell surface marker can be removed or lysed and the remaining desired mixture retained.

DETD [0414] In positive separation, ***antibodies*** specific for markers present on the desired cells of interest. For example, in the case of a TH1 cell subpopulation-related disorder wherein it would be desirable to increase the number of TH1 cells, such ***antibodies*** could be directed to the extracellular domain of the 200 gene product. Alternatively, in the case of TH2 cell subpopulation-related disorders wherein it would be desirable to increase the number of TH1 cells, such ***antibodies*** could be directed to the extracellular domain of the 103 gene product. Cells bound by the ***antibody*** are separated and retained. It will be understood that positive and negative separations can be used substantially simultaneously or in. . .

DETD [0415] A common technique for ***antibody*** based separation is the use of flow cytometry such as by a fluorescence activated cell sorter (FACS). Typically, separation by flow cytometry is performed as follows. The suspended mixture of cells are centrifuged and resuspended in media. ***Antibodies*** which are conjugated to fluorochrome are added to allow the binding of the ***antibodies*** to specific cell surface markers. The cell mixture is then washed by one or more centrifugation and resuspension steps. The. . .

DETD . . . such method is biotin-avidin based separation by affinity chromatography. Typically, such a technique is performed by incubating cells with biotin-coupled ***antibodies*** to specific markers, such as, for example, the transmembrane protein encoded by the 103 gene described herein, followed by passage through an avidin column. Biotin-***antibody***-cell complexes bind to the column via the biotin-avidin interaction, while other cells pass through the column. The specificity of the. . .

DETD . . . proliferation of the purified TH cell subpopulation. Such an expansion agent can, for example, be any appropriate cytokine, antigen, or ***antibody***. In the case of TH2 cells, for example, the expansion agent can be IL-4, while for TH1 cells, the expansion. . .

DETD [0425] Targeting moieties can include, but are not limited to, ***antibodies*** directed to cell surface markers found specifically on the TH cell subpopulation being targeted, or, alternatively, to ligands, such as. . .

DETD [0426] In the case of TH2 cells, for example, such a targeting moiety can represent an ***antibody*** directed against the extracellular portion of the 103 gene product described herein, or can, alternatively, represent a ligand specific for. . . this receptor-type TH2 specific molecule. In the case of TH1 cells, for example, such a targeting moiety can represent an ***antibody*** directed against the extracellular portion of the 200 gene product described herein, or can, alternatively, represent a ligand specific for. . .

DETD [0431] As defined herein, a therapeutically effective amount of ***antibody***, protein, or polypeptide (i.e., an effective dose or effective dosage) ranges from about 0.001 to 30 mg/kg of body weight,. . .

DETD . . . subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a protein, polypeptide or ***antibody*** can include a single treatment or, preferably, can include a series of treatments. In a preferred example,

a subject is treated with ***antibody***, protein, or polypeptide in the range of between about 0.1 to 20 mg/kg body weight one time per week for. . . even more preferably for about 4, 5, or 6 weeks. It will also be appreciated that the effective dosage of ***antibody***, protein or polypeptide used for treatment may increase or decrease over the course of a particular treatment. Changes in dosage. . .

DETD 5.11.3. Pharmaceutical Preparation and Administration of
Antibodies

DETD [0444] ***Antibodies*** which specifically bind to target gene products of the invention and thereby modulate their activity can also be administered to. . . to treat or ameliorate ischemic disorders or injuries. For example, Section 13, below, demonstrates the use of anti-200 gene product ***antibodies*** to block recovery of kidney tissue from ischemia reperfusion injury.

DETD [0445] ***Antibodies*** of the invention are administered by any suitable means, including those described in Section 5.12.2, above. In addition, ***antibody*** to a target gene product of the invention is suitably administered by pulse infusion, particularly with declining doses of the ***antibody***. Preferably, the dosing is administered by injections, most preferably by intravenous or subcutaneous injections, depending in part on whether the. . .

DETD [0446] The appropriate dosage of ***antibody*** will depend on the type of disease to be treated, the severity and course of the disease, whether the ***antibody*** is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the ***antibody***, and the discretion of the attending physician. The ***antibody*** may suitably administered to the patient at one time or, more preferably, over a series of treatments.

DETD [0447] As a general proposition, the initial pharmaceutically effective amount of ***antibody*** administered parenterally will be in the range of about 0.1 to 20 mg/kg of patient body weight per day, with. . .

DETD [0453] Such methods can, for example, utilize reagents such as the fingerprint gene nucleotide sequences described in Sections 5.1, and ***antibodies*** directed against differentially expressed and pathway gene peptides, as described, above, in Sections 5.5 (peptides) and 5.6 (***antibodies***). Specifically, such reagents can be used, for example, for: 1) the detection of the presence of target gene expression, target. . .

DETD . . . performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one specific fingerprint gene nucleic acid or anti-fingerprint gene ***antibody*** reagent described herein, which can be conveniently used, e.g., in clinical settings, to diagnose patients exhibiting TH1- or TH2-related abnormalities.

DETD . . . stimulating the cells prior to contacting the cells with the compound. Among the methods for stimulation are stimulation via anti-CD3 ***antibody*** stimulation.

DETD [0478] ***Antibodies*** directed against wild type or mutant fingerprint gene peptides, which are discussed, above, in Section 5.6, can also be used. . .

DETD . . . employed herein can, for example, be such as those described in Harlow and Lane (Harlow, E. and Lane, D., 1988, " ***Antibodies*** : A Laboratory Manual", Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.), which is incorporated herein by reference in its. . .

DETD . . . molecules can involve, for example, immunoassays wherein fingerprint gene peptides are detected by their interaction with an anti-fingerprint gene product-specific ***antibody*** .

DETD [0481] For example, ***antibodies*** , or fragments of ***antibodies*** , such as those described, above, in Section 5.6, useful in the present invention can be used to quantitatively or qualitatively. . . wild type or mutant fingerprint gene peptides. This can be accomplished, for example, by immunofluorescence techniques employing a fluorescently labeled ***antibody*** (see below, this Section,) coupled with light microscopic, flow cytometric, or fluorimetric detection. Such techniques are especially preferred if the.

DETD [0482] The ***antibodies*** (or fragments thereof) useful in the present invention can, additionally, be employed histologically, as in immunofluorescence or immunoelectron microscopy, for. . . peptides. In situ detection can be accomplished by removing a histological specimen from a patient, and applying thereto a labeled ***antibody*** of the present invention. The ***antibody*** (or fragment) is preferably applied by overlaying the labeled ***antibody*** (or fragment) onto a biological sample. Through the use of such a procedure, it is possible to determine not only. . .

DETD . . . extract, freshly harvested cells, or cells which have been incubated in tissue culture, in the presence of a detectably labeled ***antibody*** capable of identifying fingerprint gene peptides, and detecting the bound ***antibody*** by any of a number of techniques well-known in the art.

DETD . . . soluble proteins. The support can then be washed with suitable buffers followed by treatment with the detectably labeled fingerprint gene-specific ***antibody*** . The solid phase support can then be washed with the buffer a second time to remove unbound ***antibody*** . The amount of bound label on solid support can then be detected by conventional means.

DETD [0485] By "solid phase support or carrier" is intended any support capable of binding an antigen or an ***antibody*** . Well-known supports or carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, gabbros, and magnetite.. . . have virtually any possible structural configuration so long as the coupled molecule is capable of binding to an antigen or ***antibody*** . Thus, the support configuration can be spherical, as in a bead, or cylindrical, as in the inside surface of a. . . strip, etc. Preferred supports include polystyrene beads. Those skilled in the art will know many other suitable carriers for binding ***antibody*** or antigen, or will be able to ascertain the same by use of routine experimentation.

DETD [0486] The binding activity of a given lot of anti-wild type or mutant fingerprint gene product ***antibody*** can be determined according to well known methods. Those skilled in the art will be able to determine operative and. . .

DETD [0487] One of the ways in which the fingerprint gene peptide-specific ***antibody*** can be detectably labeled is by linking the same to an enzyme and use in an enzyme immunoassay (EIA) (Voller,. . . Raton, Fla.; Ishikawa, E. et al., (eds.), 1981, ENZYME IMMUNOASSAY, Kaku Shoin, Tokyo). The enzyme which is bound to the ***antibody*** will react with an appropriate substrate, preferably a chromogenic substrate, in such a manner as to produce a chemical moiety. . . be detected,

for example, by spectrophotometric, fluorimetric or by visual means. Enzymes which can be used to detectably label the ***antibody*** include, but are not limited to, malate dehydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, yeast alcohol dehydrogenase, alpha-glycerophosphate, dehydrogenase, triose phosphate isomerase, .

DETD [0488] Detection can also be accomplished using any of a variety of other immunoassays. For example, by radioactively labeling the ***antibodies*** or ***antibody*** fragments, it is possible to detect fingerprint gene wild type or mutant peptides through the use of a radioimmunoassay (RIA). . .

DETD [0489] It is also possible to label the ***antibody*** with a fluorescent compound. When the fluorescently labeled ***antibody*** is exposed to light of the proper wavelength, its presence can then be detected due to fluorescence. Among the most. . .

DETD [0490] The ***antibody*** can also be detectably labeled using fluorescence emitting metals such as .sup.152Eu, or others of the lanthanide series. These metals can be attached to the ***antibody*** using such metal chelating groups as diethylenetriaminepentacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA).

DETD [0491] The ***antibody*** also can be detectably labeled by coupling it to a chemiluminescent compound. The presence of the chemiluminescent-tagged ***antibody*** is then determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of. . .

DETD [0492] Likewise, a bioluminescent compound can be used to label the ***antibody*** of the present invention. Bioluminescence is a type of chemiluminescence found in biological systems in, which a catalytic protein increases. . .

DETD . . . samples were isolated from either unstimulated cells or from cells which had been stimulated for 6 hours with plate-bound anti-CD3 ***antibody***. The samples were probed with band 103 sequences, as shown in FIG. 6. While 103 gene RNA is present in. . .

DETD . . . E. et al., John Wiley & Sons, NY, pp 3.12.4-3.12.6). Specifically, prior to plating, the flasks were coated with anti-CD3-epsilon. ***antibodies*** (hybridoma supernatant from the 145-C11 hybridoma; Parmlingen, Inc., San Diego Calif.). For coating, ***antibodies*** were resuspended in PBS at 1-2 .mu.g/ml at a volume sufficient to coat the bottom of the flasks. Coating solution. . .

DETD [0551] After incubation, the ***antibody*** coating solution was removed by aspiration and cells were immediately added. Flasks were placed in a 37.degree. C. incubator for. . .

DETD . . . samples were isolated from either unstimulated cells (-) or cells which had been stimulated for 6 hours with plate-bound anti-CD3 ***antibody*** (+). The samples were probed with 200 gene sequences, and, as shown in FIG. 18, RNA from both stimulated and. . .

DETD . . . blot data depicted in FIG. 19 additionally illustrates 54 gene expression within cell clones either stimulated or unstimulated with anti-CD3 ***antibodies***, and demonstrates that 54 gene expression goes down within stimulated TH1 cells.

DETD . . . Specifically, RNA was isolated from unstimulated AE7 clones as well as from AE7 clones which had been stimulated with anti-CD3 ***antibodies*** for varying lengths of time, as noted in FIG. 20. As illustrated in FIG. 20, 54 gene expression decreased slightly. . .

DETD . . . herein presents in vivo data demonstrating that the 103 gene

product regulates TH2 effector cell responses. In particular, a monoclonal ***antibody*** (3E10 mAb) has been generated against the 103 gene product, and its effect in an adoptive transfer model of TH1.

DETD [0646] Rat monoclonal ***antibodies*** (MAbs), including the 3E10 MAb, were generated against the extracellular domain of the mouse 103 gene product. A DNA sequence. . .

DETD . . . culture supernatant (or 1 .mu.g purified 3E10 protein) was applied 1.times.10.sup.6 cells. After rinsing, cells were contacted with goat anti-rat ***antibody*** conjugated with PE (R-phycoerythrin) fluorescent dye. After a final rinse, cell analysis was carried out on a FACS Vantage (Becton. . .

DETD [0682] To further investigate the expression and role of the 103 gene product in TH cells, a monoclonal ***antibody*** (3E10 mAb) directed against the extracellular domain of the 103 gene product was prepared and characterized.

DETD . . . corroborating the results presented in the Example of Section 7, above. In addition, the data demonstrate the feasibility of using ***antibodies*** to separate TH2 subpopulation cells away from other cell types, thereby modulating a TH cell subpopulation by changing the number. . .

DETD . . . vivo data that the 200 gene product is involved in the recovery from kidney ischemia injury. In particular, a monoclonal ***antibody*** (96.3.8H7 mAb) generated against the extracellular domain of the murine 200 gene product, and its effect in a surgical model. . .

DETD [0703] Rat monoclonal ***antibodies*** (mAbs) were generated against the extracellular domain of the mouse 200 gene product. Recombinant murine 200 gene IgG1 (m200Ig) fusion. . .

DETD . . . For 200 gene product blockage experiments, mice were pretreated 24 hours before surgery with 100 .mu.g/mouse of rat anti-200 monoclonal ***antibody*** (200 mAb). In control experiments, mice were administered equivalent dosages of rat Ig (RtIg) ***antibody*** .

DETD [0708] Mice were then given ***antibody*** at 24 hour intervals following surgical recovery (4 hours post anesthetic).

DETD [0713] A monoclonal ***antibody*** (96.3.8H7 mAb) directed against the extracellular domain of the 200 gene product was prepared and characterized, and this ***antibody*** was administered to mice 24 hours before, and at 24 hour intervals following ischemic kidney injury. Control animals were administered. . .

DETD . . . high levels of 103 gene expression in a human mast cell line. The example also describes the production of monoclonal ***antibodies*** which are specific for the human, but not mouse, 103 gene product. FAC staining of the human mast cell line demonstrated binding of these monoclonal ***antibodies***, confirming that the 103 gene product is, indeed, expressed in mast cells.

DETD [0719] Monoclonal ***antibodies*** (MAbs; see below) were generated against the extracellular domain (amino acid residues 18-323) of a human 103 gene product (with. . .

DETD . . . protein using fluorescence activated cells sorting (FACS) according to standard methods described in Section 12.1, above using anti-mouse IgFITC secondary ***antibodies*** .

DETD . . . in a human mast cell line. Expression of the 103 gene product in this cell line was verified using monoclonal ***antibodies*** raised against an Fc fusion protein of the human 103 gene product, as

described in Section 14.1, above.

DETD [0732] FACS staining of the human mast cell line, with the 21 monoclonal ***antibodies*** showed staining with 15 of the 21 ***antibodies*** compared to isotype controls. Five of these 15 ***antibodies***, identified as 1B4, 2O3, 3F7, 3H18, and 10F7, were selected for further analysis. FAC staining with these ***antibodies*** was demonstrated to be specifically blocked with an excess of human 103-Fc fusion protein, however, staining was not blocked with. . .

DETD . . . is expressed in a human mast cell line. Accordingly, the 103 gene, its gene product, and compositions derived therefrom (e.g., ***antibodies*** and other compounds which bind to and/or modulate the expression or activity of the 103 gene or its gene product). . .

CLM What is claimed is:

. . . method for ameliorating a symptom of an ischemic disorder or injury in a mammal, comprising administering to the mammal an ***antibody*** directed against a 200 gene product in an amount effective to ameliorate the symptom of the disorder.

17. The method of claim 3 wherein said administering of the ***antibody*** is parenteral, subcutaneous, intraperitoneal, intrapulmonary, intranasal, or intralesional.

. . . 18. The method of claim 17, wherein the intralesional administration comprises perfusing or contacting a graft or organ with the ***antibody*** before transplant.

19. The method of claim 3, wherein the amount of the ***antibody*** administered is from about 1 .mu.g/kg to about 100 mg/kg.

20. The method of claim 19, wherein the amount of the ***antibody*** administered is from about 1 .mu.g/kg to about 15 mg/kg.

21. The method of claim 20, wherein the amount of the ***antibody*** administered is from about 0.1 mg/kg to about 2.0 mg/kg.

L3 ANSWER 10 OF 13 USPATFULL on STN

AN 2002:213764 USPATFULL

TI Rath genes and polypeptides and methods for the treatment and diagnosis of immune disorders

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PI US 2002115140 A1 20020822

AI US 2001-873438 A1 20010604 (9)

RLI Continuation of Ser. No. US 1997-949005, filed on 10 Oct 1997, ABANDONED

Division of Ser. No. US 1997-870815, filed on 6 Jun 1997, PATENTED

Continuation-in-part of Ser. No. US 1996-726228, filed on 4 Oct 1996,
PATENTED

DT Utility

FS APPLICATION

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CLMN Number of Claims: 57

ECL Exemplary Claim: 1

DRWN 3 Drawing Page(s)

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CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates, first, to the identification of novel nucleic acid molecules, termed RATH genes and RATH gene products encoded by such nucleic acid molecules, or degenerate variants thereof, that participate in the regulation, control and/or modulation of G-protein-mediated signal transduction involved in T cell activation, including, but not limited to T helper (TH) cell and TH cell subpopulation activation. Specifically, the nucleic acid molecules of the present invention include the genes corresponding to the mammalian RATH genes, including the RATH1.1 genes. Sequence analysis indicates that the RATH genes are novel genes belonging to the RGS ("regulator of G-protein signalling") gene family, a gene family which encodes gene products involved in G-protein-mediated signal transduction.

IN ***Levinson, Douglas Adam***, Sherborn, MA, UNITED STATES

SUMM . . . TH cells are involved in both humoral and cell-mediated forms of effector immune responses. With respect to the humoral, or ***antibody***, immune response, ***antibodies*** are produced by B lymphocytes through interactions with TH cells. In murine cells, extracellular antigens are endocytosed by antigen-presenting cells. . . complex (MHC) molecules to CD4^{sup.}+ class II MHC-restricted TH cells. These TH cells in turn activate B lymphocytes, resulting in ***antibody*** production.

SUMM . . . cells drive humoral immune responses, which are associated with, for example, defenses against certain helminthic parasites, and are involved in ***antibody*** and allergic responses.

SUMM . . . recombinant DNA molecules, cloned genes or degenerate variants thereof, especially naturally occurring variants, which encode novel RATH gene products, and ***antibodies*** directed against such RATH gene products or conserved variants or fragments thereof. The compositions of the present invention additionally include. . .

DETD . . . present invention further include RATH gene products (e.g., proteins) that are encoded by the RATH gene. Also described herein are ***antibodies*** against RATH gene products (e.g., proteins), or conserved variants or fragments thereof, and nucleic acid probes useful for the identification. . .

DETD . . . mutant allele. In this manner, gene products made by the putatively mutant tissue may be expressed and screened using standard ***antibody*** screening techniques in conjunction with ***antibodies*** raised against the normal RATH gene product, as described, below, in Section 5.3. (For screening techniques, see, for example, Harlow, E. and Lane, eds., 1988, " ***Antibodies*** : A Laboratory Manual", Cold Spring Harbor Press, Cold Spring Harbor.) In cases where a RATH mutation results in an expressed. . . altered function (e.g., as a result of a missense or a frameshift mutation), a polyclonal set of anti-RATH gene product ***antibodies*** are likely to cross-react with the mutant RATH gene product. Library clones detected via their reaction with such labeled ***antibodies*** can be purified and subjected to sequence analysis according to methods well known to those of skill in the art.

DETD . . . a variety of uses. For example, such gene products, or peptide fragments thereof, can be used for the generation of ***antibodies***, in diagnostic assays, for the identification of other cellular gene products involved in the modulation of T cell activity, such. . .

DETD . . . of such a protein is to be produced, for the generation of

pharmaceutical compositions of RATH protein or for raising
antibodies to RATH protein, vectors which direct the expression
of high levels of fusion protein products that are readily purified may.

DETD [0076] Alternatively, any fusion protein may be readily purified by
utilizing an ***antibody*** specific for the fusion protein being
expressed. For example, a system described by Janknecht et al. allows
for the ready. . .

DETD . . . from the animal, in situ hybridization analysis, and RT-PCR.
Samples of RATH gene-expressing tissue, may also be evaluated
immunocytochemically using ***antibodies*** specific for the RATH
transgene product.

DETD 5.3. ***ANTIBODIES*** TO RATH GENE PRODUCTS

DETD [0084] Described herein are methods for the production of
antibodies capable of specifically recognizing one or more RATH
gene product epitopes or epitopes of conserved variants or peptide
fragments of. . .

DETD [0085] Such ***antibodies*** may include, but are not limited to,
polyclonal ***antibodies***, monoclonal ***antibodies*** (mAbs),
humanized or chimeric ***antibodies***, single chain
antibodies, Fab fragments, F(ab').sub.2 fragments, fragments
produced by a Fab expression library, anti-idiotypic (anti-Id)
antibodies, and epitope-binding fragments of any of the above.
Such ***antibodies*** may be used, for example, in the detection of
a RATH gene product in an biological sample and may, therefore,. . .
for abnormal levels of RATH gene products, and/or for the presence of
abnormal forms of the such gene products. Such ***antibodies*** may
also be utilized in conjunction with, for example, compound screening
schemes, as described, below, in Section 5.4.2, for the evaluation of
the effect of test compounds on RATH gene product levels and/or
activity. Additionally, such ***antibodies*** can be used in
conjunction with the gene therapy techniques described, below, in
Section 5.4.3, to, for example, evaluate the. . .

DETD [0086] Anti-RATH gene product ***antibodies*** may additionally be
used as a method for the inhibition of abnormal RATH gene product
activity. Thus, such ***antibodies*** may, therefore, be utilized as
part of immune disorder treatment methods.

DETD [0087] For the production of ***antibodies*** against a RATH gene
product, various host animals may be immunized by injection with a RATH
gene product, or a. . .

DETD [0088] Polyclonal ***antibodies*** are heterogeneous populations of
antibody molecules derived from the sera of animals immunized
with an antigen, such as a RATH gene product, or an antigenic functional
derivative thereof. For the production of polyclonal ***antibodies***
, host animals such as those described above, may be immunized by
injection with RATH gene product supplemented with adjuvants as. . .

DETD [0089] Monoclonal ***antibodies***, which are homogeneous
populations of ***antibodies*** to a particular antigen, may be
obtained by any technique which provides for the production of
antibody molecules by continuous cell lines in culture. These
include, but are not limited to, the hybridoma technique of Kohler and.
. . . 4:72; Cole et al., 1983, Proc. Natl. Acad. Sci. USA 80:2026-2030),
and the EBV-hybridoma technique (Cole et al., 1985, Monoclonal
Antibodies And Cancer Therapy, Alan R. Liss, Inc., pp. 77-96).
Such ***antibodies*** may be of any immunoglobulin class including

IgG, IgM, IgE, IgA, IgD and any subclass thereof. The hybridoma producing the. . .

DETD [0090] In addition, techniques developed for the production of "chimeric ***antibodies***" (Morrison et al., 1984, Proc. Natl. Acad. Sci., 81:6851-6855; Neuberger et al., 1984, Nature, 312:604-608; Takeda et al., 1985, Nature, 314:452-454) by splicing the genes from a mouse ***antibody*** molecule of appropriate antigen specificity together with genes from a human ***antibody*** molecule of appropriate biological activity can be used. A chimeric ***antibody*** is a molecule in which different portions are derived from different animal species, such as those having a variable region. . .

DETD [0091] In addition, techniques have been developed for the production of humanized ***antibodies***. (See, e.g., Queen, U.S. Pat. No. 5,585,089, which is incorporated herein by reference in its entirety.) An immunoglobulin light or. . . "Sequences of Proteins of Immunological Interest," Kabat, E. et al., U.S. Department of Health and Human Services, 1983). Briefly, humanized ***antibodies*** are ***antibody*** molecules from non-human species having one or more CDRs from the non-human species and a framework region from a human. .

DETD [0092] Alternatively, techniques described for the production of single chain ***antibodies*** (U.S. Pat. No. 4,946,778; Bird, 1988, Science 242:423-426; Huston et al., 1988, Proc. Natl. Acad. Sci. USA 85:5879-5883; and Ward et al., 1989, Nature 334:544-546) can be adapted to produce single chain ***antibodies*** against RATH gene products. Single chain ***antibodies*** are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting. . .

DETD [0093] ***Antibody*** fragments which recognize specific epitopes may be generated by known techniques. For example, such fragments include but are not limited to: the F(ab').sub.2 fragments which can be produced by pepsin digestion of the ***antibody*** molecule and the Fab fragments which can be generated by reducing the disulfide bridges of the F(ab').sub.2 fragments. Alternatively, Fab. . .

DETD 5.4. USES OF THE RATH GENE, GENE PRODUCTS, AND ***ANTIBODIES***

DETD [0094] Described herein are various applications of the RATH genes, the RATH gene products, including peptide fragments thereof, and of ***antibodies*** directed against the RATH gene products and peptide fragments thereof.

DETD [0100] Such methods may, for example, utilize reagents such as the RATH gene nucleotide sequences described in Sections 5.1, and ***antibodies*** directed against RATH gene products, including peptide fragments thereof, as described, above, in Section 5.2. Specifically, such reagents may be. . .

DETD . . . for example, by utilizing pre-packaged diagnostic test kits comprising at least one specific RATH gene nucleic acid or anti-RATH gene ***antibody*** reagent described herein, which may be conveniently used, e.g., in clinical settings, to screen and diagnose patients exhibiting immune disorder. . .

DETD [0119] ***Antibodies*** directed against wild type or mutant RATH gene products or conserved variants or peptide fragments thereof, which are discussed, above,. . . of RATH gene product. Because evidence disclosed herein indicates that the RATH gene product is an intracellular gene product, the ***antibodies*** and immunoassay methods described below have important in vitro applications in

assessing the efficacy of treatments for immune disorders such as those described herein. ***Antibodies*** , or fragments of ***antibodies*** , such as those described below, may be used to screen potentially therapeutic compounds in vitro to determine their effects on. . .

DETD . . . efficacy of cell-based gene therapy for immune disorders involving T cell activation, including TH cell and TH cell subpopulation activation. ***Antibodies*** directed against RATH peptides may be used in vitro to determine the level of RATH gene expression achieved in cells. . .

DETD . . . employed herein may, for example, be such as those described in Harlow and Lane (Harlow, E. and Lane, D., 1988, " ***Antibodies*** : A Laboratory Manual", Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.), which is incorporated herein by reference in its. . .

DETD . . . are the result of alternatively spliced transcripts, or peptide fragments are detected by their interaction with an anti-RATH gene product-specific ***antibody*** .

DETD [0123] For example, ***antibodies*** , or fragments of ***antibodies*** , such as those described, above, in Section 5.3, useful in the present invention may be used to quantitatively or qualitatively detect the presence of RATH gene products or conserved variants or peptide fragments thereof. The ***antibodies*** (or fragments thereof) useful in the present invention may, additionally, be employed histologically, as in immunofluorescence or immunoelectron microscopy, for. . . thereof. In situ detection may be accomplished by removing a histological specimen from a patient, and applying thereto a labeled ***antibody*** of the present invention. The ***antibody*** (or fragment) is preferably applied by overlaying the labeled ***antibody*** (or fragment) onto a biological sample. Through the use of such a procedure, it is possible to determine not only. . .

DETD . . . harvested cells, or lysates of cells which have been incubated in cell culture, in the presence of a detectably labeled ***antibody*** capable of identifying RATH gene products or conserved variants or peptide fragments thereof, and detecting the bound ***antibody*** by any of a number of techniques well-known in the art.

DETD . . . proteins. The support may then be washed with suitable buffers followed by treatment with the detectably labeled RATH gene specific ***antibody*** . The solid phase support may then be washed with the buffer a second time to remove unbound ***antibody*** . The amount of bound label on solid support may then be detected by conventional means.

DETD [0126] By "solid phase support or carrier" is intended any support capable of binding an antigen or an ***antibody*** . Well-known supports or carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, gabbros, and magnetite.. . . have virtually any possible structural configuration so long as the coupled molecule is capable of binding to an antigen or ***antibody*** . Thus, the support configuration may be spherical, as in a bead, or cylindrical, as in the inside surface of a. . . strip, etc. Preferred supports include polystyrene beads. Those skilled in the art will know many other suitable carriers for binding ***antibody*** or antigen, or will be able to ascertain the same by use of routine experimentation.

DETD [0127] The binding activity of a given lot of anti-RATH gene product ***antibody*** may be determined according to well known methods.

Those skilled in the art will be able to determine operative and. . .

DETD [0128] One of the ways in which the RATH gene peptide-specific ***antibody*** can be detectably labeled is by linking the same to an enzyme and use in an enzyme immunoassay (EIA) (Voller, . . . Raton, Fla.,; Ishikawa, E. et al., (eds.), 1981, Enzyme Immunoassay, Kaku Shoin, Tokyo). The enzyme which is bound to the ***antibody*** will react with an appropriate substrate, preferably a chromogenic substrate, in such a manner as to produce a chemical moiety. . . be detected, for example, by spectrophotometric, fluorimetric or by visual means. Enzymes which can be used to detectably label the ***antibody*** include, but are not limited to, malate dehydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, yeast alcohol dehydrogenase, alpha-glycerophosphate, dehydrogenase, triose phosphate isomerase, . .

DETD [0129] Detection may also be accomplished using any of a variety of other immunoassays. For example, by radioactively labeling the ***antibodies*** or ***antibody*** fragments, it is possible to detect RATH gene peptides through the use of a radioimmunoassay (RIA) (see, for example, Weintraub, . . .

DETD [0130] It is also possible to label the ***antibody*** with a fluorescent compound. When the fluorescently labeled ***antibody*** is exposed to light of the proper wave length, its presence can then be detected due to fluorescence. Among the. . .

DETD [0131] The ***antibody*** can also be detectably labeled using fluorescence emitting metals such as .sup.152Eu, or others of the lanthanide series. These metals can be attached to the ***antibody*** using such metal chelating groups as diethylenetriaminepentacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA).

DETD [0132] The ***antibody*** also can be detectably labeled by coupling it to a chemiluminescent compound. The presence of the chemiluminescent-tagged ***antibody*** is then determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of. . .

DETD [0133] Likewise, a bioluminescent compound may be used to label the ***antibody*** of the present invention. Bioluminescence is a type of chemiluminescence found in biological systems in, which a catalytic protein increases. . .

DETD . . . may be accomplished by simply coating the solid surface with a solution of the protein and drying. Alternatively, an immobilized ***antibody***, preferably a monoclonal ***antibody***, specific for the protein to be immobilized may be used to anchor the protein to the solid surface. The surfaces. . .

DETD . . . is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; e.g., using a labeled ***antibody*** specific for the previously nonimmobilized component (the ***antibody***, in turn, may be directly labeled or indirectly labeled with a labeled anti-Ig ***antibody***).

DETD . . . be conducted in a liquid phase, the reaction products separated from unreacted components, and complexes detected; e.g., using an immobilized ***antibody*** specific for RATH gene product or the test compound to anchor any complexes formed in solution, and a labeled ***antibody*** specific for the other component of the possible complex to detect anchored complexes.

DETD . . . probing expression libraries with labeled RATH protein, using RATH protein in a manner similar to the well known technique of

antibody probing of .lambda.gt11 libraries.

DETD . . . coating the solid surface with a solution of the RATH gene product or interacting partner and drying. Alternatively, an immobilized ***antibody*** specific for the species to be anchored may be used to anchor the species to the solid surface. The surfaces. . .

DETD . . . is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; e.g., using a labeled ***antibody*** specific for the initially non-immobilized species (the ***antibody***, in turn, may be directly labeled or indirectly labeled with a labeled anti-Ig ***antibody***). Depending upon the order of addition of reaction components, test compounds which inhibit complex formation or which disrupt preformed complexes. . .

DETD . . . or absence of the test compound, the reaction products separated from unreacted components, and complexes detected; e.g., using an immobilized ***antibody*** specific for one of the interacting components to anchor any complexes formed in solution, and a labeled ***antibody*** specific for the other partner to detect anchored complexes. Again, depending upon the order of addition of reactants to the. . .

DETD . . . is maintained in the resulting fusion protein. The intracellular interacting partner can be purified and used to raise a monoclonal ***antibody***, using methods routinely practiced in the art and described above, in Section 5.2. This ***antibody*** can be labeled with the radioactive isotope .sup.125I, for example, by methods routinely practiced in the art. In a heterogeneous. . . binding, to occur. At the end of the reaction period, unbound material can be washed away, and the labeled monoclonal ***antibody*** can be added to the system and allowed to bind to the complexed components. The interaction between the RATH gene. . .

DETD . . . away. Again the extent of inhibition of the RATH gene product/interacting partner interaction can be detected by adding the labeled ***antibody*** and measuring the radioactivity associated with the beads.

DETD . . . into the tissue site, or modified antisense molecules, designed to target the desired cells (e.g., antisense linked to peptides or ***antibodies*** that specifically bind receptors or antigens expressed on the target cell surface) can be administered systemically.

DETD [0229] ***Antibodies*** exhibiting modulatory capability can be utilized to ameliorate immune disorders. Depending on the specific ***antibody***, the modulatory effect can be increase or decrease RATH activity. Such ***antibodies*** can be generated using standard techniques described in Section 5.3, above, against full length wild type or mutant RATH proteins, or against peptides corresponding to portions of the proteins. The ***antibodies*** include but are not limited to polyclonal, monoclonal, FAb fragments, single chain ***antibodies***, chimeric ***antibodies***, and the like.

DETD [0230] Because RATH is an intracellular protein, it is preferred internalizing ***antibodies*** be used. However, lipofectin or liposomes can be used to deliver the ***antibody*** or a fragment of the Fab region which binds to the RATH gene product epitope into cells. Where fragments of the ***antibody*** are used, the smallest inhibitory fragment which binds to the RATH protein's binding domain is preferred. For example, peptides having an amino acid sequence corresponding to the domain of the variable region of the ***antibody*** that binds to the RATH protein can be used. Such

peptides can be synthesized chemically or produced via recombinant DNA.

. . . methods well known in the art (e.g., see Creighton, 1983, supra; and Sambrook et al., 1989, above). Alternatively, single chain ***antibodies***, such as neutralizing ***antibodies***, which bind to intracellular epitopes can also be administered. Such single chain ***antibodies*** can be administered, for example, by expressing nucleotide sequences encoding single-chain ***antibodies*** within the target cell population by utilizing, for example, techniques such as those described in Marasco et al. (Marasco, W. . .

DETD . . . was stimulated for 6 hours by plating on plastic culture dishes which had been coated with anti-mouse CD3 epsilon monoclonal ***antibody*** (hybridoma supernatant from the 145-2C11 hybridoma; Parmingen, Inc., San Diego, Calif.) at 1.5 .mu.g/ml in PBS for one hour at. . .

DETD [0300] Western Analyses: Polyclonal ***antibodies*** were generated against-peptides of the murine RATH1.1 protein utilizing standard techniques. Specifically, the RATH peptides against which ***antibodies*** were produced were as follows (from amino to carboxy termini): DEYIRSEADKEVNID; EKDSYPRFLKSPAY; and SPAYRDLAAQASATS. Standard Western blotting techniques were utilized.

DETD [0304] Murine cell lines AE7 (TH1) and D1OG4 (TH2) were stimulated by crosslinking with an anti-CD3 monoclonal ***antibody***. At 0, 5 and overnight time points, the cells were checked for RATH protein expression using Western analysis with anti-RATH ***antibodies***. At zero hours, there was no detectable signal, while at 5 hours a clear signal was apparent, which was also. . .

DETD . . . polyacrylamide gel (Novex, San Diego, Calif.) and then transferred to an immobilon PYDF membrane (Millipore, San Francisco, Calif.). The primary ***antibody*** utilized was a rabbit anti-yeast GAL4 DNA-binding domain polyclonal ***antibody*** (Upstate Biotechnology Inc.; Lake Placid, N.Y.) and the secondary ***antibody*** was a donkey anti-rabbit Ig, peroxidase linked species-specific whole ***antibody*** (Amersham Life Science; Cleveland, Ohio). Western blotting procedures were essentially as described in Sambrook et al. (1989, Molecular Cloning, 2nd edition, Cold Spring Harbor Laboratory Press) and proteins interacting with the ***antibodies*** were visualized using the ECL detection system (Amersham Life Sciences), essentially as described by the manufacturer. Expression of the GAL4. . .

CLM What is claimed is:

25. An ***antibody*** preparation, which is specifically reactive with an epitope of a RATH polypeptide.

. . . quantitating a wild type or mutant RATH polypeptide in a sample, comprising the steps of contacting the sample with the ***antibody*** of claim 25; and detecting and/or quantitating a polypeptide-***antibody*** complex as an indication of the presence or absence and/or amount of a wildtype or mutant RATH nucleic acid.

L3 ANSWER 11 OF 13 USPATFULL on STN

AN 2002:85145 USPATFULL

TI SECRETED NEURAL ADHESION PROTEINS

IN McCarthy, Sean Anthony, Boston, MA, UNITED STATES

Gearing, David Paul, Wellesley, MA, UNITED STATES

Levinson, Douglas Adam , Sherborn, MA, UNITED STATES

PA Millennium Pharmaceuticals, Inc. (U.S. corporation)

PI US 2002045185 A1 20020418

US 6395872 B2 20020528

AI US 2001-991326 A1 20011121 (9)

RLI Division of Ser. No. US 2000-707802, filed on 7 Nov 2000, PENDING

Continuation of Ser. No. US 1999-283503, filed on 1 Apr 1999, ABANDONED

Division of Ser. No. US 1996-752307, filed on 19 Nov 1996, GRANTED, Pat.

No. US 5952171

DT Utility

FS APPLICATION

LREP MILLENNIUM PHARMACEUTICALS, INC., 75 Sidney Street, Cambridge, MA, 02139

CLMN Number of Claims: 11

ECL Exemplary Claim: 1

DRWN 10 Drawing Page(s)

LN.CNT 1537

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention features a method for identifying a cDNA nucleic acid encoding a mammalian protein having a signal sequence, which method includes the following steps:

- a) providing library of mammalian cDNA;
 - b) ligating the library of mammalian cDNA to DNA encoding alkaline phosphatase lacking both a signal sequence and a membrane anchor sequence to form ligated DNA;
 - c) transforming bacterial cells with the ligated DNA to create a bacterial cell clone library;
 - d) isolating DNA comprising the mammalian cDNA from at least one clone in the bacterial cell clone library;
 - e) separately transfecting DNA isolated from clones in step (d) into mammalian cells which do not express alkaline phosphatase to create a mammalian cell clone library wherein each clone in the mammalian cell clone library corresponds to a clone in the bacterial cell clone library;
 - f) identifying a clone in the mammalian cell clone library which express alkaline phosphatase;
 - g) identifying the clone in the bacterial cell clone library corresponding to the clone in the mammalian cell clone library identified in step (f); and
 - h) isolating and sequencing a portion of the mammalian cDNA present in the bacterial cell library clone identified in step (g) to identify a mammalian cDNA encoding a mammalian protein having a signal sequence.
- IN ***Levinson, Douglas Adam*** , Sherborn, MA, UNITED STATES
- SUMM [0033] By "purified ***antibody*** " is meant ***antibody*** which is at least 60%, by weight, free from the proteins and naturally-occurring organic molecules with which it is naturally. . . Preferably, the preparation is at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight, ***antibody*** .

SUMM [0034] By "specifically binds" is meant an ***antibody*** which recognizes and binds ethb0018f2 protein but which does not substantially recognize and bind other molecules in a sample, e.g., . . .

L3 ANSWER 12 OF 13 USPATFULL on STN

AN 2001:153109 USPATFULL

TI Compositions and methods for the treatment and diagnosis of immune disorders

IN ***Levinson, Douglas Adam*** , 111 Maple St., Sherborn, MA, United States 01770

PI US 6288218 B1 20010911

AI US 1997-937399 19970925 (8)

RLI Division of Ser. No. US 1996-609583, filed on 1 Mar 1996
Continuation-in-part of Ser. No. US 1995-487748, filed on 7 Jun 1995, now patented, Pat. No. US 5721351 Continuation-in-part of Ser. No. US 1995-398633, filed on 3 Mar 1995, now patented, Pat. No. US 6066332

DT Utility

FS GRANTED

EXNAM Primary Examiner: Swartz, Rodney P.

CLMN Number of Claims: 18

ECL Exemplary Claim: 1

DRWN 43 Drawing Figure(s); 37 Drawing Page(s)

LN.CNT 5991

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to methods and compositions for the treatment and diagnosis of immune disorders, especially T helper lymphocyte-related disorders. For example, genes which are differentially expressed within and among T helper (TH) cells and TH cell subpopulations, which include, but are not limited to TH0, TH1 and TH2 cell subpopulations are identified. Genes are also identified via the ability of their gene products to interact with gene products involved in the differentiation, maintenance and effector function of such TH cells and TH cell subpopulations. The genes identified can be used diagnostically or as targets for therapeutic intervention. In this regard, the present invention provides methods for the identification and therapeutic use of compounds as treatments of immune disorders, especially TH cell subpopulation-related disorders. Additionally, methods are provided for the diagnostic evaluation and prognosis of TH cell subpopulation-related disorders, for the identification of subjects exhibiting a predisposition to such conditions, for monitoring patients undergoing clinical evaluation for the treatment of such disorders, and for monitoring the efficacy of compounds used in clinical trials.

IN ***Levinson, Douglas Adam*** , 111 Maple St., Sherborn, MA, United States 01770

SUMM TH cells are involved in both humoral and cell-mediated forms of effector immune responses. With respect to the humoral, or ***antibody*** , immune response, ***antibodies*** are produced by B lymphocytes through interactions with TH cells. Specifically, extracellular antigens are endocytosed by antigen-presenting cells (APCs), processed, . . . complex (MHC) molecules to CD4.sup.+ class II MHC-restricted TH cells. These TH cells in turn activate B lymphocytes, resulting in ***antibody*** production.

SUMM . . . cells drive humoral immune responses, which are associated with, for example, defenses against certain helminthic parasites, and are involved in ***antibody*** and allergic responses.

SUMM The present invention also includes the products of such fingerprint, target, and pathway genes, as well as ***antibodies*** to such gene products. Furthermore, the engineering and use of cell- and animal-based models of TH cell subpopulation-related disorders to. . .

SUMM . . . of 10 gene product activity, can be particularly amenable to modulation. For example, natural ligands, derivatives of natural ligands and ***antibodies*** which bind to the 10 gene product can be utilized to reduce the number of induced T cells present by. . .

SUMM . . . product, including, for example, soluble fusion proteins such as Ig-tailed fusion proteins, can be particularly useful for this purpose. Additionally, ***antibodies*** directed against one or more of the extracellular portions of the 10 gene product may either reduce 10 gene product function by, for example, blocking ligand binding. Additionally, ***antibodies*** directed against the 10 gene product can, in certain instances, serve to increase the level of 10 gene product activity.

SUMM . . . IgE immune response, including but not limited to the symptoms which accompany atopic conditions such as allergy and/or asthma.

IgE-type ***antibodies*** are produced by stimulated B cells which require, at least in part, IL-4 produced by the TH2 cell subpopulation. Therefore,. . .

SUMM . . . one of its gene products is a membrane-bound TH2 cell subpopulation molecule. Accordingly, natural ligands, derivatives of natural ligands and ***antibodies*** which bind to this 103 gene product, can be utilized to reduce the number of TH2 cells present by either. . .

SUMM . . . cell subpopulation-restricted, but the Ig superfamily 200 gene product is, additionally, membrane-bound. Therefore, natural ligands, derivatives of natural ligands and ***antibodies*** which bind to the 200 gene product can be utilized to reduce the number of TH1 cells present by either. . .

DRWD . . . D10.G4, DAX; TH1: AE7.A, Dorris, D1.1). Clones were either unstimulated (-) or stimulated (+) for 6 hours with plate-bound anti-CD3 ***antibody***. Ten micrograms of total RNA were loaded per lane. The positions of 18s and 28s ribosomal RNA are shown as. . .

DRWD . . . 6, brain; lane 7, heart; lane 8, lung; lane 9, spleen; lane 10, liver. Clones were stimulated with plate-bound anti-CD3 ***antibody*** for 6 hours. 7.5 and 10 micrograms total RNA was used for each cell line and each tissue, respectively. a,. . .

DRWD . . . D10.G4, DAX; TH1: AE7.A, Dorris, D1.1). Clones were either unstimulated (-) or stimulated (+) for 6 hours with plate-bound anti-CD3 ***antibody***. The positions of RNA markers, in kilobases, are shown for reference. The arrow marks the position of 200 gene mRNA.

DRWD . . . (D1.1, Dorris, AE7) cell lines and TH2 (D10.G4, DAX, CDC25) cell lines, either stimulated (+) or unstimulated (-) with anti-CD3 ***antibodies***. 15 micrograms of total RNA were loaded per lane.

Cells were stimulated between 6 and 7 hours with anti-CD3

antibodies, as described, below, in Section 8.1. The Northern blots were hybridized with a probe made from the entire band 54. . .

DRWD . . . (DAX, CDC25) was isolated from either unstimulated cells or from cells which had been stimulated for two hours with anti-CD3 ***antibodies***. 15 micrograms total RNA were loaded per lane. A band 54 DNA probe was used for hybridization.

DETD Further, the gene products of such fingerprint, target, and pathway genes are described in Section 5.5, ***antibodies*** to such gene

products are described in Section 5.6, as are cell- and animal-based models of TH cell subpopulation differentiation. . .

DETD . . . limited to, pharmacological methods, such as exposure to phorbol esters, calcium ionophores, or lectins (e.g., Concanavalin A), by treatment with ***antibodies*** directed against T-cell receptor epitopes (e.g., anti-CD3 ***antibodies***) or exposure, in the presence of an appropriate antigen presenting cell (APC), to an antigen that the particular TH cells. . .

DETD . . . maintenance, and/or effector function of the subpopulations, using this protein in a manner similar to the well known technique of ***antibody*** probing of .lambda.gt11 libraries.

DETD . . . mutant allele. In this manner, gene products made by the putatively mutant tissue can be expressed and screened using standard ***antibody*** screening techniques in conjunction with ***antibodies*** raised against the normal gene product, as described, below, in Section 5.6. (For screening techniques, see, for example, Harlow, E. and Lane, eds., 1988, " ***Antibodies*** : A Laboratory Manual", Cold Spring Harbor Press, Cold Spring Harbor.) In cases where the mutation results in an expressed gene product with altered function (e.g., as a result of a missense mutation), a polyclonal set of ***antibodies*** are likely to cross-react with the mutant gene product. Library clones detected via their reaction with such labeled ***antibodies*** can be purified and subjected to sequence analysis as described in this Section, above.

DETD . . . being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of ***antibodies*** or to screen peptide libraries, for example, vectors which direct the expression of high levels of fusion protein products that. . .

DETD Alternatively, any fusion protein may be readily purified by utilizing an ***antibody*** specific for the fusion protein being expressed. For example, a system described by Janknecht et al. allows for the ready. . .

DETD Indirect labeling involves the use of a protein, such as a labeled ***antibody*** , which specifically binds to either a differentially expressed or pathway gene product. Such ***antibodies*** include but are not limited to polyclonal, monoclonal, chimeric, single chain, Fab fragments and fragments produced by an Fab expression. . .

DETD 5.6. ***Antibodies*** Specific for Differentially Expressed or Pathway Gene Products

DETD Described herein are methods for the production of ***antibodies*** capable of specifically recognizing one or more differentially expressed or pathway gene product epitopes. Such ***antibodies*** can include, but are not limited to, polyclonal ***antibodies*** , monoclonal ***antibodies*** (mAbs), humanized or chimeric ***antibodies*** , single chain ***antibodies*** , Fab fragments, F(ab').sub.2 fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) ***antibodies*** , and epitope-binding fragments of any of the above. The Ig tails of such ***antibodies*** can be modified to reduce complement activation and Fc binding. (See, for example, European Patent No. 239400 B1, Aug. 3,. . .

DETD Such ***antibodies*** can be used, for example, in the detection of a fingerprint, target, or pathway gene product in a biological sample, and can be used as part of diagnostic techniques. Alternatively, such ***antibodies*** can be utilized as part of an immune disorder treatment method, as described, below, in Section 5.9. For example, the

antibodies can be used to modulate target gene activity, can be used to modulate TH cell subpopulation differentiation, maintenance and/or effector function, or, in the case of ***antibodies*** directed to cell surface epitopes, can be used to isolate a TH cell subpopulation of interest, for either depletion or. . .

DETD For the production of ***antibodies*** to a differentially expressed or pathway gene, various host animals can be immunized by injection with a differentially expressed or. . .

DETD Polyclonal ***antibodies*** are heterogeneous populations of ***antibody*** molecules derived from the sera of animals immunized with an antigen, such as target gene product, or an antigenic functional derivative thereof. For the production of polyclonal ***antibodies***, host animals such as those described above, can be immunized by injection with differentially expressed or pathway gene product supplemented. . .

DETD Monoclonal ***antibodies***, which are homogeneous populations of ***antibodies*** to a particular antigen, can be obtained by any technique which provides for the production of ***antibody*** molecules by continuous cell lines in culture. These include, but are not limited to the hybridoma technique of Kohler and. . . 4:72; Cole et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:2026-2030), and the EBV-hybridoma technique (Cole et al., 1985, Monoclonal ***Antibodies*** And Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). Such ***antibodies*** can be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD and any subclass thereof. The hybridoma producing the. . .

DETD In addition, techniques developed for the production of "chimeric ***antibodies***" (Morrison et al., 1984, Proc. Natl. Acad. Sci., 81:6851-6855; Neuberger et al., 1984, Nature, 312:604-608; Takeda et al., 1985, Nature, 314:452-454; U.S. Pat. No. 4,816,567) by splicing the genes from a mouse ***antibody*** molecule of appropriate antigen specificity together with genes from a human ***antibody*** molecule of appropriate biological activity can be used. A chimeric ***antibody*** is a molecule in which different portions are derived from different animal species, such as those having a variable region.

DETD Alternatively, techniques described for the production of single chain ***antibodies*** (U.S. Pat. No. 4,946,778; Bird, 1988, Science 242:423-426; Huston et al., 1988, Proc. Natl. Acad. Sci. U.S.A. 85:5879-5883; and Ward et al., 1989, Nature 334:544-546) and for making humanized monoclonal ***antibodies*** (U.S. Pat. No. 5,225,539, which is incorporated herein by reference in its entirety) can be utilized to produce anti-differentially expressed or anti-pathway gene product ***antibodies***.

DETD ***Antibody*** fragments which recognize specific epitopes can be generated by known techniques. For example, such fragments include but are not limited to: the F(ab').sub.2 fragments which can be produced by pepsin digestion of the ***antibody*** molecule and the Fab fragments which can be generated by reducing the disulfide bridges of the F(ab').sub.2 fragments. Alternatively, Fab. . .

DETD ***Antibodies*** to the differentially expressed or pathway gene products can, in turn, be utilized to generate anti-idiotypic ***antibodies*** that "mimic" such gene products, using techniques well known to those skilled in the art. (See, e.g., Greenspan & Bona, . . Nissinoff, 1991, J. Immunol. 147(8):2429-2438). For example, in

the case of receptor-type molecules (e.g., 10, 103 and 200 gene products) ***antibodies*** which bind to the ECD and competitively inhibit the binding of ligand to the receptor can be used to generate.

- DET D . . . from the animal, in situ hybridization analysis, and RT-PCR. Samples of target gene-expressing tissue, can also be evaluated immunocytochemically using ***antibodies*** specific for the target gene transgene gene product of interest.
- DET D The target gene transgenic animals that express target gene mRNA or target gene transgene peptide (detected immunocytochemically, using ***antibodies*** directed against target gene product epitopes) at easily detectable levels can then be further evaluated to identify those animals which. . .
- DET D . . . limited to members of random or partially degenerate, directed phosphopeptide libraries; see, e.g., Songyang, Z. et al., 1993, Cell 72:767-778), ***antibodies*** (including, but not limited to polyclonal, monoclonal, humanized, anti-idiotypic, chimeric or single chain ***antibodies***, and FAb, F(ab').sub.2 and FAb expression library fragments, and epitope-binding fragments thereof), and small organic or inorganic molecules. In the. . . bind to the ECD and either mimic the activity triggered by the natural ligand (i.e., agonists); as well as peptides, ***antibodies*** or fragments thereof, and other organic compounds that mimic the ECD (or a portion thereof) and bind to a "neutralize". . .
- DET D . . . can be accomplished by simply coating the solid surface with a solution of the protein and drying. Alternatively, an immobilized ***antibody***, preferably a monoclonal ***antibody***, specific for the protein to be immobilized can be used to anchor the protein to the solid surface. The surfaces. . .
- DET D . . . is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; e.g., using a labeled ***antibody*** specific for the previously nonimmobilized component (the ***antibody***, in turn, can be directly labeled or indirectly labeled with a labeled anti-Ig ***antibody***).
- DET D . . . be conducted in a liquid phase, the reaction products separated from unreacted components, and complexes detected; e.g., using an immobilized ***antibody*** specific for target gene product or the test compound to anchor any complexes formed in solution, and a labeled ***antibody*** specific for the other component of the possible complex to detect anchored complexes.
- DET D . . . target gene protein, especially mutant target gene proteins. Such compounds can include, but are not limited to molecules such as ***antibodies***, peptides, and the like, as described, for example, in Section 5.8.1. above.
- DET D . . . coating the solid surface with a solution of the target gene product or binding partner and drying. Alternatively, an immobilized ***antibody*** specific for the species to be anchored can be used to anchor the species to the solid surface. The surfaces. . .
- DET D . . . is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; e.g., using a labeled ***antibody*** specific for the initially non-immobilized species (the ***antibody***, in turn, can be directly labeled or indirectly labeled with a labeled anti-Ig ***antibody***). Depending upon the order of addition of reaction components, test compounds which inhibit complex formation or which disrupt preformed complexes. . .

DETD . . . or absence of the test compound, the reaction products separated from unreacted components, and complexes detected; e.g., using an immobilized ***antibody*** specific for one of the binding components to anchor any complexes formed in solution, and a labeled ***antibody*** specific for the other partner to detect anchored complexes. Again, depending upon the order of addition of reactants to the. . .

DETD . . . the resulting fusion protein. The interactive cellular or extracellular binding partner can be purified and used to raise a monoclonal ***antibody***, using methods routinely practiced in the art and described above, in Section 5.6. This ***antibody*** can be labeled with the radioactive isotope ¹²⁵I, for example, by methods routinely practiced in the art. In a. . . binding to occur. At the end of the reaction period, unbound material can be washed away, and the labeled monoclonal ***antibody*** can be added to the system and allowed to bind to the complexed components. The interaction between the target gene. . .

DETD . . . away. Again the extent of inhibition of the target gene product/binding partner interaction can be detected by adding the labeled ***antibody*** and measuring the radioactivity associated with the beads.

DETD . . . limited to members of random or partially degenerate, directed phosphopeptide libraries; see, e.g., Songyang, Z. et al., 1993, Cell 72:767-778), ***antibodies*** (including, but not limited to polyclonal, monoclonal, humanized, anti-idiotypic, chimeric or single chain ***antibodies***, and FAb, F(ab').sub.2 and FAb expression library fragments, and epitope-binding fragments thereof), and small organic or inorganic molecules.

DETD . . . soluble Ig-tailed fusion proteins which may be produced by utilizing techniques such as those described, above, in Section 5.5. Additionally, ***antibodies*** directed against the extracellular portion of the 10 gene product may reduce 10 gene product function by, for example, blocking. . .

DETD . . . for example, peptides representing soluble extracellular portions of target gene product transmembrane receptors), phosphopeptides, small organic or inorganic molecules, or ***antibodies*** (including, for example, polyclonal, monoclonal, humanized, anti-idiotypic, chimeric or single chain ***antibodies***, and FAb, F(ab').sub.2 and FAb expression library fragments, and epitope-binding fragments thereof). Techniques for the determination of effective doses and. . .

DETD . . . IgE immune responses, including, but not limited to the symptoms which accompany atopic conditions such as allergy and/or asthma. IgE-type ***antibodies*** are produced by stimulated B cells which require, at least in part, IL-4 produced by the TH2 cell subpopulation. Therefore,. . .

DETD . . . a reduction in the activity and/or effective concentration of TH2 cells. For example, natural ligands, derivatives of natural ligands and ***antibodies*** which bind to the 103 gene product can be utilized to reduce the number of TH2 cells present by either. . .

DETD . . . a reduction in the activity and/or effective concentration of TH1 cells. For example, natural ligands, derivatives of natural ligands and ***antibodies*** which bind to the 200 gene product can be utilized to reduce the number of TH1 cells present by either. . .

DETD . . . and/or analogs thereof, of the gene 200 product, including, for

example, soluble fusion proteins such as Ig-tailed fusion proteins or ***antibodies***. (For a discussion of the production of Ig-tailed fusion proteins see, for example, U.S. Pat. No. 5,116,964.)

DETD . . . these 200 gene product domains or mutants fused to another polypeptide (e.g., an IgFc polypeptide) can be utilized. Alternatively, anti-idiotypic ***antibodies*** or Fab fragments of antiidiotypic ***antibodies*** that mimic the 200 gene product ECD and neutralize 200 gene product ligand can be used. Such 200 gene product peptides, proteins, fusion proteins, anti-idiotypic ***antibodies*** or Fabs are administered to a subject in amounts sufficient to neutralize Ob and to effectuate an amelioration of a . . .

DETD . . . into the tissue site, or modified antisense molecules, designed to target the desired cells (e.g., antisense linked to peptides or ***antibodies*** that specifically bind receptors or antigens expressed on the target cell surface) can be administered systemically.

DETD . . . limited to peptides representing soluble extracellular portions of target gene product transmembrane proteins, phosphopeptides, small organic or inorganic molecules, or ***antibodies*** (including, for example, polyclonal, monoclonal, humanized, anti-idiotypic, chimeric or single chain ***antibodies***, and FAb, F(ab').sub.2 and FAb expression library fragments, and epitope-binding fragments thereof).

DETD 5.9.3.1. ***Antibody*** Techniques

DETD ***Antibodies*** exhibiting modulatory capability can be utilized to ameliorate immune disorders such as TH cell subpopulation-related disorders. Depending on the specific ***antibody***, the modulatory effect can be negative and can, therefore, be utilized as part of the techniques described, above, in Section. . .

DETD An ***antibody*** having negative modulatory capability refers to an ***antibody*** which specifically binds to and interferes with the action of a protein. In the case of an extracellular receptor, for example, such an ***antibody*** would specifically bind the extracellular domain of the receptor in a manner which does not activate the receptor but which disrupts the ability of the receptor to bind its natural ligand. For example, ***antibodies*** directed against the extracellular domains of genes 103 or 200 can function as such negative modulators. Additionally, ***antibodies*** directed against one or more of the 10 gene product extracellular domains can function in a negative modulatory manner. Such ***antibodies*** can be generated using standard techniques described in Section 5.6, above, against full length wild type or mutant proteins, or against peptides corresponding to portions of the proteins. The ***antibodies*** include but are not limited to polyclonal, monoclonal, FAb fragments, single chain ***antibodies***, chimeric ***antibodies***, and the like.

DETD An ***antibody*** having positive modulatory capability refers to an ***antibody*** which specifically binds to a protein and, by binding, serves to, either directly or indirectly, activate the function of the protein which it recognizes. For example, an ***antibody*** can bind to the extracellular portion of a transmembrane protein in a manner which causes the transmembrane protein to function as though its endogenous ligand was binding, thus activating, for example, a signal transduction pathway. ***antibodies*** can be generated using standard techniques described in Section 5.6, above, against full length wild type or mutant proteins, or against peptides corresponding to portions of the proteins. The ***antibodies*** include but are not limited to polyclonal, monoclonal, FAb fragments, single chain

antibodies , chimeric ***antibodies*** , and the like.

DETD In instances where the protein, such as a target gene protein, to which the ***antibody*** is directed is intracellular and whole ***antibodies*** are used, internalizing ***antibodies*** can be preferred. However, lipofectin or liposomes can be used to deliver the ***antibody*** or a fragment of the Fab region which binds to the gene product epitope into cells. Where fragments of the ***antibody*** are used, the smallest inhibitory fragment which binds to the protein's binding domain is preferred. For example, peptides having an amino acid sequence corresponding to the domain of the variable region of the ***antibody*** that binds to the protein can be used. Such peptides can be synthesized chemically or produced via recombinant DNA technology. . . methods well known in the art (e.g., see Creighton, 1983, supra; and Sambrook et al., 1989, above). Alternatively, single chain ***antibodies*** , such as neutralizing ***antibodies*** , which bind to intracellular epitopes can also be administered. Such single chain ***antibodies*** can be administered, for example, by expressing nucleotide sequences encoding single-chain ***antibodies*** within the target cell population by utilizing, for example, techniques such as those described in Marasco et al. (Marasco, W. . .

DETD In instances where the protein to which the ***antibody*** is directed is extracellular, or is a transmembrane protein, any of the administration techniques described, below in Section 5.10 which are appropriate for peptide administration can be utilized to effectively administer the ***antibodies*** to their site of action.

DETD . . . of the separation is to increase or augment the number of cells belonging to a specific TH cell subpopulation, the ***antibodies*** used can also be specific to surface markers present on undifferentiated or partially undifferentiated TH cells. After separation, and purification. . .

DETD . . . such as TH1 or TH2 cells, can be removed, and thus selectively separated and purified, by various methods which utilize ***antibodies*** which bind specific markers present on the T cell population of interest, while absent on other cells within the starting. . . a fluorescence activated cell sorter (FACS) and specific fluorochromes, biotin-avidin or biotin-streptavidin separations using biotin conjugated to cell surface marker-specific ***antibodies*** and avidin or streptavidin bound to a solid support such as affinity column matrix or plastic surfaces or magnetic separations using ***antibody*** -coated magnetic beads.

DETD Separation via ***antibodies*** for specific markers can be by negative or positive selection procedures. In negative separation, ***antibodies*** are used which are specific for markers present on undesired cells. For example, in the case of a TH1 cell subpopulation-related disorder wherein it would be desirable to deplete the number of TH1 cells, such ***antibodies*** could be directed to the extracellular domain of the 200 gene product. Alternatively, in the case of TH2 cell subpopulation-related disorders wherein it would be desirable to deplete the number of TH1 cells, such ***antibodies*** could be directed to the extracellular domain of the 103 gene product. Cells bound by an ***antibody*** to such a cell surface marker can be removed or lysed and the remaining desired mixture retained.

DETD In positive separation, ***antibodies*** specific for markers present on the desired cells of interest. For example, in the case of a TH1 cell subpopulation-related disorder wherein it would be desirable to

increase the number of TH1 cells, such ***antibodies*** could be directed to the extracellular domain of the 200 gene product. Alternatively, in the case of TH2 cell subpopulation-related disorders wherein it would be desirable to increase the number of TH1 cells, such ***antibodies*** could be directed to the extracellular domain of the 103 gene product. Cells bound by the ***antibody*** are separated and retained. It will be understood that positive and negative separations can be used substantially simultaneously or in. . .

DETD A common technique for ***antibody*** based separation is the use of flow cytometry such as by a fluorescence activated cell sorter (FACS). Typically, separation by flow cytometry is performed as follows. The suspended mixture of cells are centrifuged and resuspended in media. ***Antibodies*** which are conjugated to fluorochrome are added to allow the binding of the ***antibodies*** to specific cell surface markers. The cell mixture is then washed by one or more centrifugation and resuspension steps. The. . .

DETD . . . such method is biotin-avidin based separation by affinity chromatography. Typically, such a technique is performed by incubating cells with biotin-coupled ***antibodies*** to specific markers, such as, for example, the transmembrane protein encoded by the 103 gene described herein, followed by passage through an avidin column. Biotin-***antibody*** -cell complexes bind to the column via the biotin-avidin interaction, while other cells pass through the column. The specificity of the. . .

DETD . . . proliferation of the purified TH cell subpopulation. Such an expansion agent can, for example, be any appropriate cytokine, antigen, or ***antibody***. In the case of TH2 cells, for example, the expansion agent can be IL-4, while for TH1 cells, the expansion. . .

DETD Targeting moieties can include, but are not limited to, ***antibodies*** directed to cell surface markers found specifically on the TH cell subpopulation being targeted, or, alternatively, to ligands, such as. . .

DETD In the case of TH2 cells, for example, such a targeting moiety can represent an ***antibody*** directed against the extracellular portion of the 103 gene product described herein, or can, alternatively, represent a ligand specific for. . . this receptor-type TH2 specific molecule. In the case of TH1 cells, for example, such a targeting moiety can represent an ***antibody*** directed against the extracellular portion of the 200 gene product described herein, or can, alternatively, represent a ligand specific for. . .

DETD Such methods can, for example, utilize reagents such as the fingerprint gene nucleotide sequences described in Sections 5.1, and ***antibodies*** directed against differentially expressed and pathway gene peptides, as described, above, in Sections 5.5 (peptides) and 5.6 (***antibodies***). Specifically, such reagents can be used, for example, for: 1) the detection of the presence of target gene expression, target. . .

DETD . . . performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one specific fingerprint gene nucleic acid or anti-fingerprint gene ***antibody*** reagent described herein, which can be conveniently used, e.g., in clinical settings, to diagnose patients exhibiting TH1- or TH2-related abnormalities.

DETD ***Antibodies*** directed against wild type or mutant fingerprint gene peptides, which are discussed, above, in Section 5.6, can also be used. . .

DETD . . . employed herein can, for example, be such as those described in Harlow and Lane (Harlow, E. and Lane, D., 1988, " ***Antibodies*** : A Laboratory Manual", Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.), which is incorporated herein by reference in its. . .

DETD . . . molecules can involve, for example, immunoassays wherein fingerprint gene peptides are detected by their interaction with an anti-fingerprint gene product-specific ***antibody*** .

DETD For example, ***antibodies*** , or fragments of ***antibodies*** , such as those described, above, in Section 5.6, useful in the present invention can be used to quantitatively or qualitatively. . . wild type or mutant fingerprint gene peptides. This can be accomplished, for example, by immunofluorescence techniques employing a fluorescently labeled ***antibody*** (see below, this Section,) coupled with light microscopic, flow cytometric, or fluorimetric detection. Such techniques are especially preferred if the. . .

DETD The ***antibodies*** (or fragments thereof) useful in the present invention can, additionally, be employed histologically, as in immunofluorescence or immunoelectron microscopy, for. . . peptides. In situ detection can be accomplished by removing a histological specimen from a patient, and applying thereto a labeled ***antibody*** of the present invention. The ***antibody*** (or fragment) is preferably applied by overlaying the labeled ***antibody*** (or fragment) onto a biological sample. Through the use of such a procedure, it is possible to determine not only. . .

DETD . . . extract, freshly harvested cells, or cells which have been incubated in tissue culture, in the presence of a detectably labeled ***antibody*** capable of identifying fingerprint gene peptides, and detecting the bound ***antibody*** by any of a number of techniques well-known in the art.

DETD . . . soluble proteins. The support can then be washed with suitable buffers followed by treatment with the detectably labeled fingerprint gene-specific ***antibody*** . The solid phase support can then be washed with the buffer a second time to remove unbound ***antibody*** . The amount of bound label on solid support can then be detected by conventional means.

DETD By "solid phase support or carrier" is intended any support capable of binding an antigen or an ***antibody*** . Well-known supports or carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, gabbros, and magnetite.. . . have virtually any possible structural configuration so long as the coupled molecule is capable of binding to an antigen or ***antibody*** . Thus, the support configuration can be spherical, as in a bead, or cylindrical, as in the inside surface of a. . . strip, etc. Preferred supports include polystyrene beads. Those skilled in the art will know many other suitable carriers for binding ***antibody*** or antigen, or will be able to ascertain the same by use of routine experimentation.

DETD The binding activity of a given lot of anti-wild type or mutant fingerprint gene product ***antibody*** can be determined according to well known methods. Those skilled in the art will be able to determine operative and. . .

DETD One of the ways in which the fingerprint gene peptide-specific ***antibody*** can be detectably labeled is by linking the same to an enzyme and use in an enzyme immunoassay (EIA) (Voller,. . . Raton, Fla.; Ishikawa, E. et al., (eds.), 1981, ENZYME IMMUNOASSAY, Kaku

Shoin, Tokyo). The enzyme which is bound to the ***antibody*** will react with an appropriate substrate, preferably a chromogenic substrate, in such a manner as to produce a chemical moiety. . . be detected, for example, by spectrophotometric, fluorimetric or by visual means. Enzymes which can be used to detectably label the ***antibody*** include, but are not limited to, malate dehydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, yeast alcohol dehydrogenase, alpha-glycerophosphate, dehydrogenase, triose phosphate isomerase, . .

DETD Detection can also be accomplished using any of a variety of other immunoassays. For example, by radioactively labeling the ***antibodies*** or ***antibody*** fragments, it is possible to detect fingerprint gene wild type or mutant peptides through the use of a radioimmunoassay (RIA). . .

DETD It is also possible to label the ***antibody*** with a fluorescent compound. When the fluorescently labeled ***antibody*** is exposed to light of the proper wavelength, its presence can then be detected due to fluorescence. Among the most. . .

DETD The ***antibody*** can also be detectably labeled using fluorescence emitting metals such as .sup.152 Eu, or others of the lanthanide series. These metals can be attached to the ***antibody*** using such metal chelating groups as diethylenetriaminepentacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA).

DETD The ***antibody*** also can be detectably labeled by coupling it to a chemiluminescent compound. The presence of the chemiluminescent-tagged ***antibody*** is then determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of. . .

DETD Likewise, a bioluminescent compound can be used to label the ***antibody*** of the present invention. Bioluminescence is a type of chemiluminescence found in biological systems in, which a catalytic protein increases. . .

DETD . . . samples were isolated from either unstimulated cells or from cells which had been stimulated for 6 hours with plate-bound anti-CD3 ***antibody***. The samples were probed with band 103 sequences, as shown in FIG. 6. While 103 gene RNA is present in. . .

DETD . . . et al., John Wiley & Sons, New York, pp 3.12.4-3.12.6). Specifically, prior to plating, the flasks were coated with anti-CD3-epsilon. ***antibodies*** (hybridoma supernatant from the 145-C11 hybridoma; Parmingen, Inc., San Diego Calif.). For coating, ***antibodies*** were resuspended in PBS at 1-2 .mu.g/ml at a volume sufficient to coat the bottom of the flasks. Coating solution. . .

DETD After incubation, the ***antibody*** coating solution was removed by aspiration and cells were immediately added. Flasks were placed in a 37.degree. C. incubator for. . .

DETD . . . samples were isolated from either unstimulated cells (-) or cells which had been stimulated for 6 hours with plate-bound anti-CD3 ***antibody*** (+). The samples were probed with 200 gene sequences, and, as shown in FIG. 18, RNA from both stimulated and. . .

DETD . . . blot data depicted in FIG. 19 additionally illustrates 54 gene expression within cell clones either stimulated or unstimulated with anti-CD3 ***antibodies***, and demonstrates that 54 gene expression goes down within stimulated TH1 cells.

DETD . . . Specifically, RNA was isolated from unstimulated AE7 clones as well as from AE7 clones which had been stimulated with anti-CD3

antibodies for varying lengths of time, as noted in FIG. 20. As illustrated in FIG. 20, 54 gene expression decreased slightly. . .

CLM What is claimed is:

. . . of claim 3, wherein the 200 gene product is detected in the sample by binding the gene product to an ***antibody*** directed against the 200 gene product.

6. The method of claim 5, wherein the ***antibody*** is directly or indirectly labeled.

. . . the level of the 200 gene product is detected in the patient sample by binding the gene product to an ***antibody*** directed against the 200 gene product.

15. The method of claim 14, wherein the ***antibody*** is directly or indirectly labeled.

L3 ANSWER 13 OF 13 USPATFULL on STN

AN 1998:154095 USPATFULL

TI Murine RATH gene

IN ***Levinson, Douglas Adam***, Sherborn, MA, United States

Gimeno, Carlos J., Boston, MA, United States

PA Millennium Pharmaceuticals, Inc., Cambridge, MA, United States (U.S. corporation)

PI US 5846780 19981208

AI US 1996-726228 19961004 (8)

DT Utility

FS Granted

EXNAM Primary Examiner: LeGuyader, John L.; Assistant Examiner: Shibuya, Mark L.

LREP Pennie & Edmonds LLP

CLMN Number of Claims: 19

ECL Exemplary Claim: 1

DRWN 3 Drawing Figure(s); 3 Drawing Page(s)

LN.CNT 3233

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates, first, to the identification of novel nucleic acid molecules and proteins encoded by such nucleic acid molecules, or degenerate variants thereof, that participate in the regulation, control and/or modulation of G-protein-mediated signal transduction involved in T cell activation, including, but not limited to T helper (TH) cell and TH cell subpopulation activation. Specifically, the nucleic acid molecules of the present invention include the genes corresponding to the mammalian R11 gene, including the human R11 gene. R11 sequence analysis indicates that the R11 gene is a novel gene belonging to the RGS ("regulator of G-protein signalling") gene family, a gene family which encodes gene products involved in G-protein-mediated signal transduction.

IN ***Levinson, Douglas Adam***, Sherborn, MA, United States

SUMM . . . TH cells are involved in both humoral and cell-mediated forms of effector immune responses. With respect to the humoral, or ***antibody***, immune response, ***antibodies*** are produced by B lymphocytes through interactions with TH cells. In murine cells, extracellular antigens are endocytosed by antigen-presenting cells. .

. complex (MHC) molecules to CD4^{sup.}+ class II MHC-restricted TH cells. These TH cells in turn activate B lymphocytes, resulting in ***antibody*** production.

SUMM . . . cells drive humoral immune responses, which are associated with, for example, defenses against certain helminthic parasites, and are involved in ***antibody*** and allergic responses.

SUMM . . . recombinant DNA molecules, cloned genes or degenerate variants thereof, especially naturally occurring variants, which encode novel RATH gene products, and ***antibodies*** directed against such RATH gene products or conserved variants or fragments thereof. The compositions of the present invention additionally include. . .

DETD . . . present invention further include RATH gene products (e.g., proteins) that are encoded by the RATH gene. Also described herein are ***antibodies*** against RATH gene products (e.g., proteins), or conserved variants or fragments thereof, and nucleic acid probes useful for the identification. . .

DETD . . . mutant allele. In this manner, gene products made by the putatively mutant tissue may be expressed and screened using standard ***antibody*** screening techniques in conjunction with ***antibodies*** raised against the normal RATH gene product, as described, below, in Section 5.3. (For screening techniques, see, for example, Harlow, . . . altered function (e.g., as a result of a missense or a frameshift mutation), a polyclonal set of anti-RATH gene product ***antibodies*** are likely to cross-react with the mutant RATH gene product. Library clones detected via their reaction with such labeled ***antibodies*** can be purified and subjected to sequence analysis according to methods well known to those of skill in the art.

DETD . . . a variety of uses. For example, such gene products, or peptide fragments thereof, can be used for the generation of ***antibodies***, in diagnostic assays, or for the identification of other cellular gene products involved in the modulation of T cell activity, . . .

DETD . . . of such a protein is to be produced, for the generation of pharmaceutical compositions of RATH protein or for raising ***antibodies*** to RATH protein, vectors which direct the expression of high levels of fusion protein products that are readily purified may.

DETD Alternatively, any fusion protein may be readily purified by utilizing an ***antibody*** specific for the fusion protein being expressed. For example, a system described by Janknecht et al. allows for the ready. . .

DETD . . . from the animal, in situ hybridization analysis, and RT-PCR. Samples of RATH gene-expressing tissue, may also be evaluated immunocytochemically using ***antibodies*** specific for the RATH transgene product.

DETD 5.3. ***ANTIBODIES*** TO RATH GENE PRODUCTS

DETD Described herein are methods for the production of ***antibodies*** capable of specifically recognizing one or more RATH gene product epitopes or epitopes of conserved variants or peptide fragments of. . .

DETD Such ***antibodies*** may include, but are not limited to, polyclonal ***antibodies***, monoclonal ***antibodies*** (mAbs), humanized or chimeric ***antibodies***, single chain ***antibodies***, Fab fragments, F(ab')₂ fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) ***antibodies***, and epitope-binding fragments of any of the above.

Such ***antibodies*** may be used, for example, in the detection of a RATH gene product in a biological sample and may, therefore, . . . for abnormal levels of RATH gene products, and/or for the presence of abnormal forms of the such gene products. Such ***antibodies*** may also be utilized in conjunction with, for example, compound screening schemes, as described, below, in Section 5.4.2, for the evaluation of the effect of test compounds on RATH gene product levels and/or activity. Additionally, such ***antibodies*** can be used in conjunction with the gene therapy techniques described, below, in Section 5.4.3, to, for example, evaluate the. . .

DETD Anti-RATH gene product ***antibodies*** may additionally be used as a method for the inhibition of abnormal RATH gene product activity. Thus, such ***antibodies*** may, therefore, be utilized as part of immune disorder treatment methods.

DETD For the production of ***antibodies*** against a RATH gene product, various host animals may be immunized by injection with a RATH gene product, or a. . .

DETD Polyclonal ***antibodies*** are heterogeneous populations of ***antibody*** molecules derived from the sera of animals immunized with an antigen, such as a RATH gene product, or an antigenic functional derivative thereof. For the production of polyclonal ***antibodies***, host animals such as those described above, may be immunized by injection with RATH gene product supplemented with adjuvants as. . .

DETD Monoclonal ***antibodies***, which are homogeneous populations of ***antibodies*** to a particular antigen, may be obtained by any technique which provides for the production of ***antibody*** molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique of Kohler and. . . 4:72; Cole et al., 1983, Proc. Natl. Acad. Sci. USA 80:2026-2030), and the EBV-hybridoma technique (Cole et al., 1985, Monoclonal ***Antibodies*** And Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). Such ***antibodies*** may be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD and any subclass thereof. The hybridoma producing the. . .

DETD In addition, techniques developed for the production of "chimeric ***antibodies***" (Morrison et al., 1984, Proc. Natl. Acad. Sci., 81:6851-6855; Neuberger et al., 1984, Nature, 312:604-608; Takeda et al., 1985, Nature, 314:452-454) by splicing the genes from a mouse ***antibody*** molecule of appropriate antigen specificity together with genes from a human ***antibody*** molecule of appropriate biological activity can be used. A chimeric ***antibody*** is a molecule in which different portions are derived from different animal species, such as those having a variable region. . .

DETD Alternatively, techniques described for the production of single chain ***antibodies*** (U.S. Pat. No. 4,946,778; Bird, 1988, Science 242:423-426; Huston et al., 1988, Proc. Natl. Acad. Sci. USA 85:5879-5883; and Ward et al., 1989, Nature 334:544-546) can be adapted to produce single chain ***antibodies*** against RATH gene products. Single chain ***antibodies*** are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting. . .

DETD ***Antibody*** fragments which recognize specific epitopes may be generated by known techniques. For example, such fragments include but are not limited to: the F(ab')₂ fragments which can be produced by pepsin digestion of the ***antibody*** molecule and the Fab

fragments which can be generated by reducing the disulfide bridges of the F(ab')₂ fragments. Alternatively, Fab. . .

DETD 5.4. USES OF THE RATH GENE, GENE PRODUCTS, AND ***ANTIBODIES***

DETD Described herein are various applications of the RATH gene, the RATH gene product including peptide fragments thereof, and of ***antibodies*** directed against the RATH gene product and peptide fragments thereof. Such applications include, for example, prognostic and diagnostic evaluation of. . .

DETD Such methods may, for example, utilize reagents such as the RATH gene nucleotide sequences described in Sections 5.1, and ***antibodies*** directed against RATH gene products, including peptide fragments thereof, as described, above, in Section 5.2. Specifically, such reagents may be. . .

DETD . . . for example, by utilizing pre-packaged diagnostic test kits comprising at least one specific RATH gene nucleic acid or anti-RATH gene ***antibody*** reagent described herein, which may be conveniently used, e.g., in clinical settings, to screen and diagnose patients exhibiting immune disorder. . .

DETD ***Antibodies*** directed against wild type or mutant RATH gene products or conserved variants or peptide fragments thereof, which are discussed, above,. . . of RATH gene product. Because evidence disclosed herein indicates that the RATH gene product is an intracellular gene product, the ***antibodies*** and immunoassay methods described below have important in vitro applications in assessing the efficacy of treatments for immune disorders such as those described herein. ***Antibodies***, or fragments of ***antibodies***, such as those described below, may be used to screen potentially therapeutic compounds in vitro to determine their effects on. . .

DETD . . . efficacy of cell-based gene therapy for immune disorders involving T cell activation, including TH cell and TH cell subpopulation activation. ***Antibodies*** directed against RATH peptides may be used in vitro to determine the level of RATH gene expression achieved in cells. . .

DETD . . . employed herein may, for example, be such as those described in Harlow and Lane (Harlow, E. and Lane, D., 1988, " ***Antibodies*** : A Laboratory Manual", Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.), which is incorporated herein by reference in its. . .

DETD . . . are the result of alternatively spliced transcripts, or peptide fragments are detected by their interaction with an anti-RATH gene product-specific ***antibody***.

DETD For example, ***antibodies***, or fragments of ***antibodies***, such as those described, above, in Section 5.3, useful in the present invention may be used to quantitatively or qualitatively detect the presence of RATH gene products or conserved variants or peptide fragments thereof. The ***antibodies*** (or fragments thereof) useful in the present invention may, additionally, be employed histologically, as in immunofluorescence or immunoelectron microscopy, for. . . thereof. In situ detection may be accomplished by removing a histological specimen from a patient, and applying thereto a labeled ***antibody*** of the present invention. The ***antibody*** (or fragment) is preferably applied by overlaying the labeled ***antibody*** (or fragment) onto a biological sample. Through the use of such a procedure, it is possible to determine not only. . .

DETD . . . harvested cells, or lysates of cells which have been incubated

in cell culture, in the presence of a detectably labeled
antibody capable of identifying RATH gene products or conserved
variants or peptide fragments thereof, and detecting the bound
antibody by any of a number of techniques well-known in the art.

DETD . . . proteins. The support may then be washed with suitable buffers
followed by treatment with the detectably labeled RATH gene specific
antibody. The solid phase support may then be washed with the
buffer a second time to remove unbound, ***antibody***. The amount
of bound label on solid support may then be detected by conventional
means.

DETD By "solid phase support or carrier" is intended any support capable of
binding an antigen or an ***antibody***. Well-known supports or
carriers include glass, polystyrene, polypropylene, polyethylene,
dextran, nylon, amylases, natural and modified celluloses,
polyacrylamides, gabbros, and magnetite.. . . have virtually any
possible structural configuration so long as the coupled molecule is
capable of binding to an antigen or ***antibody***. Thus, the
support configuration may be spherical, as in a bead, or cylindrical, as
in the inside surface of a . . . strip, etc. Preferred supports
include polystyrene beads. Those skilled in the art will know many other
suitable carriers for binding ***antibody*** or antigen, or will be
able to ascertain the same by use of routine experimentation.

DETD The binding activity of a given lot of anti-RATH gene product
antibody may be determined according to well known methods.
Those skilled in the art will be able to determine operative and. . .

DETD One of the ways in which the RATH gene peptide-specific ***antibody***
can be detectably labeled is by linking the same to an enzyme and use in
an enzyme immunoassay (EIA) (Voller, . . . Raton, Fla.; Ishikawa, E.
et al., (eds.), 1981, Enzyme Immunoassay, Kaku Shoin, Tokyo). The
enzyme which is bound to the ***antibody*** will react with an
appropriate substrate, preferably a chromogenic substrate, in such a
manner as to produce a chemical moiety. . . be detected, for example,
by spectrophotometric, fluorimetric or by visual means. Enzymes which
can be used to detectably label the ***antibody*** include, but are
not limited to, malate dehydrogenase, staphylococcal nuclease,
delta-5-steroid isomerase, yeast alcohol dehydrogenase,
alpha-glycerophosphate, dehydrogenase, triose phosphate isomerase,. . .

DETD Detection may also be accomplished using any of a variety of other
immunoassays. For example, by radioactively labeling the
antibodies or ***antibody*** fragments, it is possible to
detect RATH gene peptides through the use of a radioimmunoassay (RIA)
(see, for example, Weintraub,. . .

DETD It is also possible to label the ***antibody*** with a fluorescent
compound. When the fluorescently labeled ***antibody*** is exposed
to light of the proper wave length, its presence can then be detected
due to fluorescence. Among the. . .

DETD The ***antibody*** can also be detectably labeled using fluorescence
emitting metals such as .sup.152 Eu, or others of the lanthanide series.
These metals can be attached to the ***antibody*** using such metal
chelating groups as diethylenetriaminepentacetic acid (DTPA) or
ethylenediaminetetraacetic acid (EDTA).

DETD The ***antibody*** also can be detectably labeled by coupling it to
a chemiluminescent compound. The presence of the hemiluminescent-tagged
antibody is then determined by detecting the presence of

luminescence that arises during the course of a chemical reaction.

Examples of. . .

DETD Likewise, a bioluminescent compound may be used to label the ***antibody*** of the present invention. Bioluminescence is a type of chemiluminescence found in biological systems in, which a catalytic protein increases. . .

DETD . . . may be accomplished by simply coating the solid surface with a solution of the protein and drying. Alternatively, an immobilized ***antibody***, preferably a monoclonal ***antibody***, specific for the protein to be immobilized may be used to anchor the protein to the solid surface. The surfaces. . .

DETD . . . is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; e.g., using a labeled ***antibody*** specific for the previously nonimmobilized component (the ***antibody***, in turn, may be directly labeled or indirectly labeled with a labeled anti-Ig ***antibody***).

DETD . . . be conducted in a liquid phase, the reaction products separated from unreacted components, and complexes detected; e.g., using an immobilized ***antibody*** specific for RATH gene product or the test compound to anchor any complexes formed in solution, and a labeled ***antibody*** specific for the other component of the possible complex to detect anchored complexes.

DETD . . . probing expression libraries with labeled RATH protein, using RATH protein in a manner similar to the well known technique of ***antibody*** probing of .lambda.gt11 libraries.

DETD . . . coating the solid surface with a solution of the RATH gene product or interacting partner and drying. Alternatively, an immobilized ***antibody*** specific for the species to be anchored may be used to anchor the species to the solid surface. The surfaces. . .

DETD . . . is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; e.g., using a labeled ***antibody*** specific for the initially non-immobilized species (the ***antibody***, in turn, may be directly labeled or indirectly labeled with a labeled anti-Ig ***antibody***). Depending upon the order of addition of reaction components, test compounds which inhibit complex formation or which disrupt preformed complexes. . .

DETD . . . or absence of the test compound, the reaction products separated from unreacted components, and complexes detected; e.g., using an immobilized ***antibody*** specific for one of the interacting components to anchor any complexes formed in solution, and a labeled ***antibody*** specific for the other partner to detect anchored complexes. Again, depending upon the order of addition of reactants to the. . .

DETD . . . is maintained in the resulting fusion protein. The intracellular interacting partner can be purified and used to raise a monoclonal ***antibody***, using methods routinely practiced in the art and described above, in Section 5.2. This ***antibody*** can be labeled with the radioactive isotope ¹²⁵I, for example, by methods routinely practiced in the art. In a. . . binding, to occur. At the end of the reaction period, unbound material can be washed away, and the labeled monoclonal ***antibody*** can be added to the system and allowed to bind to the complexed components. The interaction between the RATH gene. . .

DETD . . . away. Again the extent of inhibition of the RATH gene product/interacting partner interaction can be detected by adding the

labeled ***antibody*** and measuring the radioactivity associated with the beads.

DETD . . . into the tissue site, or modified antisense molecules, designed to target the desired cells (e.g., antisense linked to peptides or ***antibodies*** that specifically bind receptors or antigens expressed on the target cell surface) can be administered systemically.

DETD ***Antibodies*** exhibiting modulatory capability can be utilized to ameliorate immune disorders. Depending on the specific ***antibody***, the modulatory effect can be increase or decrease RATH activity. Such ***antibodies*** can be generated using standard techniques described in Section 5.3, above, against full length wild type or mutant RATH proteins, or against peptides corresponding to portions of the proteins. The ***antibodies*** include but are not limited to polyclonal, monoclonal, Fab fragments, single chain ***antibodies***, chimeric ***antibodies***, and the like.

DETD Because RATH is an intracellular protein, it is preferred internalizing ***antibodies*** be used. However, lipofectin or liposomes can be used to deliver the ***antibody*** or a fragment of the Fab region which binds to the RATH gene product epitope into cells. Where fragments of the ***antibody*** are used, the smallest inhibitory fragment which binds to the RATH protein's binding domain is preferred. For example, peptides having an amino acid sequence corresponding to the domain of the variable region of the ***antibody*** that binds to the RATH protein can be used. Such peptides can be synthesized chemically or produced via recombinant DNA. . . methods well known in the art (e.g., see Creighton, 1983, supra; and Sambrook et al., 1989, above). Alternatively, single chain ***antibodies***, such as neutralizing ***antibodies***, which bind to intracellular epitopes can also be administered. Such single chain ***antibodies*** can be administered, for example, by expressing nucleotide sequences encoding single-chain ***antibodies*** within the target cell population by utilizing, for example, techniques such as those described in Marasco et al. (Marasco, W.. . .

DETD . . . was stimulated for 6 hours by plating on plastic culture dishes which had been coated with anti-mouse CD3 epsilon monoclonal ***antibody*** (hybridoma supernatant from the 145- 2C11 hybridoma; Parmingen, Inc., San Diego Calif.) at 1.5 .mu.g/ml in PBS for one hour.

=> e lloyd clare/au

E1	20	LLOYD CHRISTOPHER T/AU
E2	2	LLOYD CINDY/AU
E3	25	--> LLOYD CLARE/AU
E4	47	LLOYD CLARE M/AU
E5	1	LLOYD CLARENCE R/AU
E6	1	LLOYD CLARENCE ROBERT/AU
E7	1	LLOYD CLARKE B/AU
E8	2	LLOYD CLAUD A/AU
E9	1	LLOYD CLIFFORD L/AU
E10	1	LLOYD CLINTON/AU
E11	9	LLOYD CLINTON T/AU
E12	23	LLOYD CLIVE/AU

=> s e3-e4

L4 72 ("LLOYD CLARE"/AU OR "LLOYD CLARE M"/AU)

=> dup rem l4

PROCESSING COMPLETED FOR L4

L5 45 DUP REM L4 (27 DUPLICATES REMOVED)

=> s l5 and antibod?

L6 10 L5 AND ANTIBOD?

=> d bib ab kwic l-

YOU HAVE REQUESTED DATA FROM 10 ANSWERS - CONTINUE? Y/(N):y

L6 ANSWER 1 OF 10 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN

AN 2000:3188 BIOSIS

DN PREV200000003188

TI Crucial role of the interleukin 1 receptor family member T1/ST2 in T helper cell type 2-mediated lung mucosal immune responses.

AU Coyle, Anthony J. [Reprint author]; ***Lloyd, Clare*** [Reprint author]; Tian, Jane [Reprint author]; Nguyen, Trang [Reprint author]; Eriksson, Christina; Wang, Lin [Reprint author]; Ottoson, Par; Persson, Per; Delaney, Tracy [Reprint author]; Lehar, Sophie [Reprint author]; Lin, Steve [Reprint author]; Poisson, Louis [Reprint author]; Meisel, Christian; Kamradt, Thomas; Bjerke, Torbjorn; Levinson, Douglas [Reprint author]; Gutierrez-Ramos, Jose Carlos [Reprint author]

CS Department of Biology, Inflammation Division, Millennium Pharmaceuticals, Inc., Cambridge, MA, 02139, USA

SO Journal of Experimental Medicine, (Oct. 4, 1999) Vol. 190, No. 7, pp. 895-902. print.

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DT Article

LA English

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AB T1/ST2 is an orphan receptor of unknown function that is expressed on the surface of murine T helper cell type 2 (Th2), but not Th1 effector cells. In vitro blockade of T1/ST2 signaling with an immunoglobulin (Ig) fusion protein suppresses both differentiation to and activation of Th2, but not Th1 effector populations. In a nascent Th2-dominated response, anti-T1/ST2 monoclonal ***antibody*** (mAb) inhibited eosinophil infiltration, interleukin 5 secretion, and IgE production. To determine if these effects were mediated by a direct effect on Th2 cells, we next used a murine adoptive transfer model of Th1- and Th2-mediated lung mucosal immune responses. Administration of either T1/ST2 mAb or T1/ST2-Ig abrogated Th2 cytokine production in vivo and the induction of an eosinophilic inflammatory response, but failed to modify Th1-mediated inflammation. Taken together, our data demonstrate an important role of T1/ST2 in Th2-mediated inflammatory responses and suggest that T1/ST2 may prove to be a novel target for the selective suppression of Th2 immune responses.

AU Coyle, Anthony J. [Reprint author]; ***Lloyd, Clare*** [Reprint author]; Tian, Jane [Reprint author]; Nguyen, Trang [Reprint author]; Eriksson, Christina; Wang, Lin [Reprint author]; Ottoson, Par; Persson, .

AB. . . suppresses both differentiation to and activation of Th2, but not Th1 effector populations. In a nascent Th2-dominated response,

anti-T1/ST2 monoclonal ***antibody*** (mAb) inhibited eosinophil infiltration, interleukin 5 secretion, and IgE production. To determine if these effects were mediated by a direct. . .

L6 ANSWER 2 OF 10 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN

AN 1998:180867 BIOSIS

DN PREV199800180867

TI The chemotactic cytokine eotaxin acts as a granulocyte-macrophage colony-stimulating factor during lung inflammation.

AU Peled, Amnon; Gonzalo, Jose Angel; ***Lloyd, Clare*** ; Gutierrez-Ramos, Jose-Carlos [Reprint author]

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SO Blood, (March 15, 1998) Vol. 91, No. 6, pp. 1909-1916. print.

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ED Entered STN: 20 Apr 1998

Last Updated on STN: 20 Apr 1998

AB During inflammatory processes, inflamed tissues signal the bone marrow (BM) to produce more mature leukocytes in ways that are not yet understood. We report here that, during the development of lung allergic inflammation, the administration of neutralizing ***antibodies*** to the chemotactic cytokine, Eotaxin, prevented the increase in the number of myeloid progenitors produced in the BM, therefore reducing the output of mature myeloid cells from BM. Conversely, the in vivo administration of Eotaxin increased the number of myeloid progenitors present in the BM. Furthermore, we found that, in vitro, Eotaxin is a colony-stimulating factor for granulocytes and macrophages. Eotaxin activity synergized with stem cell factor but not with interleukin-3 or granulocyte-macrophage colony-stimulating factor and was inhibited by pertussis toxin. We report also that CCR-3, the receptor for Eotaxin, was expressed by hematopoietic progenitors (HP). Thus, during inflammation, Eotaxin acts in a paracrine way to shift the differentiation of BM HP towards the myeloid lineage.

AU Peled, Amnon; Gonzalo, Jose Angel; ***Lloyd, Clare*** ; Gutierrez-Ramos, Jose-Carlos [Reprint author]

AB. . . that are not yet understood. We report here that, during the development of lung allergic inflammation, the administration of neutralizing ***antibodies*** to the chemotactic cytokine, Eotaxin, prevented the increase in the number of myeloid progenitors produced in the BM, therefore reducing. . .

L6 ANSWER 3 OF 10 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN

AN 1997:20403 BIOSIS

DN PREV199799319606

TI Eosinophil recruitment to the lung in a murine model of allergic inflammation: The role of T cells, chemokines, and adhesion receptors.

AU Gonzalo, Jose-Angel; ***Lloyd, Clare M.*** ; Kremer, Leonor; Finger, Elizabeth; Martinez-A., C.; Siegelman, M. H.; Cybulsky, Myron; Gutierrez-Ramos, Jose-Carlos [Reprint author]

CS Cent. Blood Res. Incorp., Harvard Med. Sch., 200 Longwood Ave., Boston, MA 02115, USA

SO Journal of Clinical Investigation, (1996) Vol. 98, No. 10, pp. 2332-2345.

CODEN: JCINAO. ISSN: 0021-9738.

DT Article

LA English

ED Entered STN: 15 Jan 1997

Last Updated on STN: 15 Jan 1997

AB Eosinophil accumulation is a distinctive feature of lung allergic inflammation. Here, we have used a mouse model of OVA (ovalbumin)-induced pulmonary eosinophilia to study the cellular and molecular mechanisms for this selective recruitment of eosinophils to the airways. In this model there was an early accumulation of infiltrating monocytes/macrophages in the lung during the OVA treatment, whereas the increase in infiltrating T-lymphocytes paralleled the accumulation of eosinophils. The kinetics of accumulation of these three leukocyte subtypes correlated with the levels of mRNA expression of the chemokines monocyte chemoattractant peptide-1/JE, eotaxin, and RANTES (regulated upon activation in normal T cells expressed and secreted), suggesting their involvement in the recruitment of these leukocytes. Furthermore, blockade of eotaxin with specific ***antibodies*** in vivo reduced the accumulation of eosinophils in the lung in response to OVA by half. Mature CD4+ T-lymphocytes were absolutely required for OVA-induced eosinophil accumulation since lung eosinophilia was prevented in CD4+deficient mice. However, these cells were neither the main producers of the major eosinophilic chemokines eotaxin, RANTES, or MIP-1-alpha, nor did they regulate the expression of these chemokines. Rather, the presence of CD4+ T cells was necessary for enhancement of VCAM-1 (vascular cell adhesion molecule-1) expression in the lung during allergic inflammation induced by the OVA treatment. In support of this, mice genetically deficient for VCAM-1 and intercellular adhesion molecule-1 failed to develop pulmonary eosinophilia. Selective eosinophilic recruitment during lung allergic inflammation results from a sequential accumulation of certain leukocyte types, particularly T cells, and relies on the presence of both eosinophilic chemoattractants and adhesion receptors.

AU Gonzalo, Jose-Angel; ***Lloyd, Clare M.*** ; Kremer, Leonor; Finger, Elizabeth; Martinez-A., C.; Siegelman, M. H.; Cybulsky, Myron; Gutierrez-Ramos, Jose-Carlos [Reprint author]

AB. . . T cells expressed and secreted), suggesting their involvement in the recruitment of these leukocytes. Furthermore, blockade of eotaxin with specific ***antibodies*** in vivo reduced the accumulation of eosinophils in the lung in response to OVA by half. Mature CD4+ T-lymphocytes were. . .

L6 ANSWER 4 OF 10 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN

AN 1997:20261 BIOSIS

DN PREV199799319464

TI Distinct expression and function of the novel mouse chemokine monocyte chemoattractant protein-5 in lung allergic inflammation.

AU Jia, Gui-Quan; Gonzalo, Jose A.; ***Lloyd, Clare*** ; Kremer, L.; Lu, Lei; Martinez-A., C.; Wershil, B. K.; Gutierrez-Ramos, J. C. [Reprint author]

CS Center Blood Res., Inc., Harvard Med. Sch., 200 Longwood Ave., Boston, MA 02115, USA

SO Journal of Experimental Medicine, (1996) Vol. 184, No. 5, pp. 1939-1951.

CODEN: JEMEAU. ISSN: 0022-1007.

DT Article

LA English

ED Entered STN: 15 Jan 1997

Last Updated on STN: 15 Jan 1997

AB We have cloned a novel mouse CC chemokine cDNA from the lung during an allergic inflammatory reaction. The protein encoded by this cDNA is chemotactic for eosinophils, monocytes, and lymphocytes in vitro and in vivo. Based on its similarities in sequence and function with other CC chemokines, we have named it mouse monocyte chemotactic protein-5 (mMCP-5). Under noninflammatory conditions, expression of mMCP-5 in the lymph nodes and thymus is constitutive and is generally restricted to stromal cells. Neutralization of mMCP-5 protein with specific ***antibodies*** during an allergic inflammatory reaction in vivo resulted in a reduction in the number of eosinophils that accumulated in the lung. Moreover, mMCP-5 mRNA expression in vivo is regulated differently from that of other major CC chemokines in the lung during the allergic reaction, including Eotaxin. The presence of lymphocytes is essential for expression of mMCP-5 by alveolar macrophages and smooth muscle cells in the lung, and the induction of mMCP-5 RNA occurs earlier than that of the eosinophil chemokine Eotaxin during allergic inflammation. In contrast to Eotaxin, mRNA for mMCP-5 can be produced by mast cells. From these results, we postulate that mMCP-5 plays a pivotal role during the early stages of allergic lung inflammation.

AU Jia, Gui-Quan; Gonzalo, Jose A.; ***Lloyd, Clare*** ; Kremer, L.; Lu, Lei; Martinez-A., C.; Wershil, B. K.; Gutierrez-Ramos, J. C. [Reprint author]

AB. . . the lymph nodes and thymus is constitutive and is generally restricted to stromal cells. Neutralization of mMCP-5 protein with specific ***antibodies*** during an allergic inflammatory reaction in vivo resulted in a reduction in the number of eosinophils that accumulated in the. . .

L6 ANSWER 5 OF 10 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN

AN 1994:272054 BIOSIS

DN PREV199497285054

TI Characterization and pathological significance of monoclonal DNA-binding ***antibodies*** from mice with experimental malaria infection.

AU ***Lloyd, Clare M.*** ; Collins, Ian; Belcher, Alison J.; Manuelpillai, Neil; Wozencraft, Anne O.; Staines, Norman A. [Reprint author]

CS Infection Immunity Research Group, King's Coll. London, Campden Hill Road, London W8 7AH, UK

SO Infection and Immunity, (1994) Vol. 62, No. 5, pp. 1982-1988.

CODEN: INFIBR. ISSN: 0019-9567.

DT Article

LA English

ED Entered STN: 24 Jun 1994

Last Updated on STN: 24 Jun 1994

AB Malaria infection is accompanied by the production of a number of autoantibodies, including some that react with DNA. Epidemiological evidence implicates these in the nephritides that arise in human quartan malaria and in experimental malaria infections in mice. Through parallels with the involvement of DNA-reactive ***antibodies*** in the autoimmune syndrome systemic lupus erythematosus, a role for DNA-reactive ***antibodies*** in forming phlogistic immune deposits in the kidneys is implied. To more fully understand the relationship between ***antibodies*** of this specificity made in malaria and systemic lupus erythematosus, we prepared monoclonal DNA-reactive ***antibodies*** from BALB/c mice infected with Plasmodium berghei (clone RC) and compared their properties with those of other ***antibodies*** previously

isolated from lupous MRL/Mp lpr/lpr and (NZB X NZW)F-1 mice.

Antibodies from malarial mice were all immunoglobulin M class and bound to single-stranded but not double-stranded DNA in an enzyme-linked immunosorbent assay. They also reacted with synthetic polyribonucleotides in the enzyme-linked immunosorbent assay and with parasitized erythrocytes and parasite pigment in kidney sections. None of the ***antibodies*** from lupous mice had identical specificities. The potential involvement of the DNA-reactive ***antibodies*** in malarial nephritis was demonstrated, by use of immunocytochemical methods, on the basis of their binding to existing immune deposits in kidney sections from malarial mice, a similar property having been previously demonstrated for

antibodies from lupous mice. Furthermore, ***antibodies*** from malarial mice expressed public idiotypes, notably Id.V-88, which is a member of the Id.16/6 family, commonly found on DNA-reactive ***antibodies*** in lupus and other infectious and connective tissue diseases. This study indicates that DNA-reactive ***antibodies*** in malaria have immunochemical properties similar but not identical to those of such ***antibodies*** in systemic lupus erythematosus and that they have the potential to participate in the formation of immune deposits in nephritic malarial kidneys.

TI Characterization and pathological significance of monoclonal DNA-binding ***antibodies*** from mice with experimental malaria infection.

AU ***Lloyd, Clare M.*** ; Collins, Ian; Belcher, Alison J.; Manuelpillai, Neil; Wozencraft, Anne O.; Staines, Norman A. [Reprint author]

AB. . . that arise in human quartan malaria and in experimental malaria infections in mice. Through parallels with the involvement of DNA-reactive ***antibodies*** in the autoimmune syndrome systemic lupus erythematosus, a role for DNA-reactive ***antibodies*** in forming phlogistic immune deposits in the kidneys is implied. To more fully understand the relationship between ***antibodies*** of this specificity made in malaria and systemic lupus erythematosus, we prepared monoclonal DNA-reactive ***antibodies*** from BALB/c mice infected with Plasmodium berghei (clone RC) and compared their properties with those of other ***antibodies*** previously isolated from lupous MRL/Mp lpr/lpr and (NZB X NZW)F-1 mice. ***Antibodies*** from malarial mice were all immunoglobulin M class and bound to single-stranded but not double-stranded DNA in an enzyme-linked immunosorbent. . . synthetic polyribonucleotides in the enzyme-linked immunosorbent assay and with parasitized erythrocytes and parasite pigment in kidney sections. None of the ***antibodies*** from lupous mice had identical specificities. The potential involvement of the DNA-reactive ***antibodies*** in malarial nephritis was demonstrated, by use of immunocytochemical methods, on the basis of their binding to existing immune deposits in kidney sections from malarial mice, a similar property having been previously demonstrated for ***antibodies*** from lupous mice. Furthermore, ***antibodies*** from malarial mice expressed public idiotypes, notably Id.V-88, which is a member of the Id.16/6 family, commonly found on DNA-reactive ***antibodies*** in lupus and other infectious and connective tissue diseases. This study indicates that DNA-reactive ***antibodies*** in malaria have immunochemical properties similar but not identical to those of such ***antibodies*** in systemic lupus erythematosus and that they have the potential to participate in the formation of immune deposits in nephritic. . .

AN 2003:835772 CAPLUS

DN 139:322141

TI CCR4 blockade does not inhibit allergic airways inflammation

AU Conroy, Dolores M.; Jopling, Louise A.; ***Lloyd, Clare M.*** ; Hodge, Martin R.; Andrew, David P.; Williams, Timothy J.; Pease, James E.; Sabroe, Ian

CS Leukocyte Biology Section, Biomedical Sciences Division, Faculty of Medicine, Imperial College London, UK

SO Journal of Leukocyte Biology (2003), 74(4), 558-563

CODEN: JLBIE7; ISSN: 0741-5400

PB Federation of American Societies for Experimental Biology

DT Journal

LA English

AB The CC chemokine receptor 4 (CCR4) shows selectivity for the recruitment of memory T cell subsets, including those of the T helper cell type 2 (Th2) phenotype. In humans, CCR4+ T cells are recruited to the asthmatic lung in response to allergen challenge; however, the contribution of this pathway to allergic disease remains uncertain. We therefore investigated the role of CCR4 in allergic airways inflammation in the guinea pig. Blockade of CCR4 with a specific ***antibody*** resulted in only minor changes in nos. of CCR4+ Th cells in the bronchoalveolar lavage fluid of allergen-challenged guinea pigs and failed to inhibit the generation of eotaxin/CC chemokine ligand (CCL)11 or macrophage-derived chemokine/CCL22 or the recruitment of inflammatory leukocytes to the lung. These data suggest that although CCR4 was originally proposed as a marker of Th2 status, antigen-specific Th2 cells are recruited to the lung predominantly by other pathways. This study casts doubts on the validity of CCR4 as a therapeutic target in the treatment of asthma.

RE.CNT 26 THERE ARE 26 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

AU Conroy, Dolores M.; Jopling, Louise A.; ***Lloyd, Clare M.*** ; Hodge, Martin R.; Andrew, David P.; Williams, Timothy J.; Pease, James E.; Sabroe, Ian

AB The CC chemokine receptor 4 (CCR4) shows selectivity for the recruitment of memory T cell subsets, including those of the T helper cell type 2 (Th2) phenotype. In humans, CCR4+ T cells are recruited to the asthmatic lung in response to allergen challenge; however, the contribution of this pathway to allergic disease remains uncertain. We therefore investigated the role of CCR4 in allergic airways inflammation in the guinea pig. Blockade of CCR4 with a specific ***antibody*** resulted in only minor changes in nos. of CCR4+ Th cells in the bronchoalveolar lavage fluid of allergen-challenged guinea pigs and failed to inhibit the generation of eotaxin/CC chemokine ligand (CCL)11 or macrophage-derived chemokine/CCL22 or the recruitment of inflammatory leukocytes to the lung. These data suggest that although CCR4 was originally proposed as a marker of Th2 status, antigen-specific Th2 cells are recruited to the lung predominantly by other pathways. This study casts doubts on the validity of CCR4 as a therapeutic target in the treatment of asthma.

L6 ANSWER 7 OF 10 CAPLUS COPYRIGHT 2003 ACS on STN

AN 2001:101309 CAPLUS

DN 134:173895

TI Protein and cDNA sequences encoding G protein-coupled receptor 15571, which is related to the secretin-like family, and uses thereof in drug screening, diagnostic, and therapeutic applications

IN Hodge, Martin R.; ***Lloyd, Clare*** ; Weich, Nadine S.

PA Millennium Pharmaceuticals, Inc., USA

SO PCT Int. Appl., 145 pp.

CODEN: PIXXD2

DT Patent

LA English

FAN.CNT 1

PATENT NO. KIND DATE APPLICATION NO. DATE

PI WO 2001009328 A1 20010208 WO 2000-US21278 20000803

WO 2001009328 C2 20020808

WO 2001009328 C1 20031023

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH,
CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR,
KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ,
NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR,
TT, TZ, UA, UG, US, VZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD,
RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY,
DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ,
CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

EP 1208196 A1 20020529 EP 2000-952484 20000803

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
IE, SI, LT, LV, FI, RO, MK, CY, AL

JP 2003506042 T2 20030218 JP 2001-514120 20000803

PRAI US 1999-146916P P 19990803

US 2000-515781 A 20000229

WO 2000-US21278 W 20000803

AB The invention provides protein and cDNA sequences encoding a novel G protein-coupled receptor (15571), which is a new member of the secretin-like family. The invention further relates to methods using receptor polypeptides and polynucleotides as a target for diagnosis and treatment in secretin-like receptor-mediated disorders. The invention further relates to drug-screening methods using the receptor polypeptides and polynucleotides to identify agonists and antagonists for diagnosis and treatment. The invention further encompasses agonists and antagonists based on the receptor polypeptides and polynucleotides.

RE.CNT 2 THERE ARE 2 CITED REFERENCES AVAILABLE FOR THIS RECORD

ALL CITATIONS AVAILABLE IN THE RE FORMAT

IN Hodge, Martin R.; ***Lloyd, Clare*** ; Weich, Nadine S.

IT ***Antibodies***

Primers (nucleic acid)

Probes (nucleic acid)

RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses)
(protein and cDNA sequences encoding G protein-coupled receptor 15571,
which is related to secretin-like family, and uses thereof in drug
screening, diagnostic, and therapeutic applications)

L6 ANSWER 8 OF 10 CAPLUS COPYRIGHT 2003 ACS on STN

AN 2000:861842 CAPLUS

DN 134:28454

TI T helper cell-expressed polynucleotide and polypeptide compositions and
methods for the treatment and diagnosis of immune disorders

IN Levinson, Douglas A.; ***Lloyd, Clare M.*** ; McCarthy, Sean A.

PA Millennium Pharmaceuticals, Inc., USA

SO PCT Int. Appl., 311 pp.

CODEN: PIXXD2

DT Patent

LA English

FAN.CNT 5

PATENT NO. KIND DATE APPLICATION NO. DATE

PI WO 2000073498 A1 20001207 WO 2000-US14986 20000531

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR,
CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU,
ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU,
LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE,
SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW,
AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY,
DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ,
CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRAI US 1999-324986 A 19990602

AB The present invention relates to methods and compns. for the treatment and diagnosis of immune disorders, esp. T helper lymphocyte-related disorders, and also for the treatment of mast cell-related processes and disorders, ischemic disorders and injuries, including ischemic renal disorders and injuries. For example, genes which are differentially expressed within and among T helper (TH) cells and TH cell subpopulations, which include, but are not limited to TH0, TH1 and TH2 cell subpopulations are identified. Genes are also identified via the ability of their gene products to interact with gene products involved in the differentiation, maintenance and effector function of such TH cells and TH cell subpopulations. The genes identified can be used diagnostically or as targets for therapeutic intervention. In this regard, the present invention provides methods for the identification and therapeutic use of compds. as treatments of immune disorders, esp. TH cell subpopulation-related disorders. Addnl., methods are provided for the diagnostic evaluation and prognosis of TH cell subpopulation-related disorders, for the identification of subjects exhibiting a predisposition to such conditions, for monitoring patients undergoing clin. evaluation for the treatment of such disorders, and for monitoring the efficacy of compds. used in clin. trials. Methods are also provided for the treatment of symptoms assocd. with mast cell-related processes or disorders and ischemic disorders and injuries using the genes, gene products and ***antibodies*** of the invention.

RE.CNT 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

IN Levinson, Douglas A.; ***Lloyd, Clare M.*** ; McCarthy, Sean A.

AB The present invention relates to methods and compns. for the treatment and diagnosis of immune disorders, esp. T helper lymphocyte-related disorders, and also for the treatment of mast cell-related processes and disorders, ischemic disorders and injuries, including ischemic renal disorders and injuries. For example, genes which are differentially expressed within and among T helper (TH) cells and TH cell subpopulations, which include, but are not limited to TH0, TH1 and TH2 cell subpopulations are identified. Genes are also identified via the ability of their gene products to interact with gene products involved in the differentiation, maintenance and effector function of such TH cells and TH cell

subpopulations. The genes identified can be used diagnostically or as targets for therapeutic intervention. In this regard, the present invention provides methods for the identification and therapeutic use of compds. as treatments of immune disorders, esp. TH cell subpopulation-related disorders. Addnl., methods are provided for the diagnostic evaluation and prognosis of TH cell subpopulation-related disorders, for the identification of subjects exhibiting a predisposition to such conditions, for monitoring patients undergoing clin. evaluation for the treatment of such disorders, and for monitoring the efficacy of compds. used in clin. trials. Methods are also provided for the treatment of symptoms assocd. with mast cell-related processes or disorders and ischemic disorders and injuries using the genes, gene products and ***antibodies*** of the invention.

L6 ANSWER 9 OF 10 CAPLUS COPYRIGHT 2003 ACS on STN

AN 1990:530470 CAPLUS

DN 113:130470

TI Role of DNA-binding ***antibodies*** in kidney pathology associated with murine malaria infections

AU Wozencraft, Anne O.; ***Lloyd, Clare M.*** ; Staines, Norman A.; Griffiths, Verity J.

CS Div. Biomol. Sci., King's Coll., London, W8 7AH, UK

SO Infection and Immunity (1990), 58(7), 2156-64

CODEN: INFIBR; ISSN: 0019-9567

DT Journal

LA English

AB A series of studies were performed to examine the sequential development of nephritis during murine malaria infections and to define the role of DNA-binding ***antibodies*** in the assocd. pathol. Serum levels of these ***antibodies*** were assessed throughout acute and chronic malaria infections. Increased levels of double-stranded DNA- and single-stranded DNA-binding ***antibodies*** were initially detected in mice infected with Plasmodium vinckei or P. yoelii nigeriensis during the middle stages of infection, and these levels were maintained until death. Infection with the more chronic organism P. berghei clone RC also resulted in increased single-stranded DNA-binding ***antibody*** titers, which fluctuated as the infection progressed. All three species caused kidney damage and dysfunction, as assessed by changes in morphol., blood urea nitrogen, and excreted albumin; this damage correlated with the extent of parasitemia and was obsd. before the levels of DNA-binding ***antibodies*** were detectably elevated in the serum. However, the results of immunohistochem. studies demonstrated that DNA-binding monoclonal ***antibodies*** bound ex vivo to glomeruli within kidneys prep'd. from mice at late stages of infection, after the initial damage had been incurred. These findings suggest how DNA-binding ***antibodies*** could contribute to the kidney pathol. assocd. with both malaria and certain autoimmune diseases, such as systemic lupus erythematosus.

TI Role of DNA-binding ***antibodies*** in kidney pathology associated with murine malaria infections

AU Wozencraft, Anne O.; ***Lloyd, Clare M.*** ; Staines, Norman A.; Griffiths, Verity J.

AB A series of studies were performed to examine the sequential development of nephritis during murine malaria infections and to define the role of DNA-binding ***antibodies*** in the assocd. pathol. Serum levels of these ***antibodies*** were assessed throughout acute and chronic

malaria infections. Increased levels of double-stranded DNA- and single-stranded DNA-binding ***antibodies*** were initially detected in mice infected with *Plasmodium vinckei* or *P. yoelii nigeriensis* during the middle stages of infection, and these levels were maintained until death. Infection with the more chronic organism *P. berghei* clone RC also resulted in increased single-stranded DNA-binding ***antibody*** titers, which fluctuated as the infection progressed. All three species caused kidney damage and dysfunction, as assessed by changes in morphol., blood urea nitrogen, and excreted albumin; this damage correlated with the extent of parasitemia and was obsd. before the levels of DNA-binding ***antibodies*** were detectably elevated in the serum. However, the results of immunohistochem. studies demonstrated that DNA-binding monoclonal ***antibodies*** bound ex vivo to glomeruli within kidneys prep'd. from mice at late stages of infection, after the initial damage had been incurred. These findings suggest how DNA-binding ***antibodies*** could contribute to the kidney pathol. assocd. with both malaria and certain autoimmune diseases, such as systemic lupus erythematosus.

ST malaria DNA ***antibody*** nephritis

IT ***Antibodies***

RL: BIOL (Biological study)

(auto-, to DNA, in malaria-assocd. nephritis)

L6 ANSWER 10 OF 10 USPATFULL on STN

AN 2003:100085 USPATFULL

TI Compositions and methods for the treatment and diagnosis of immune disorders

IN Levinson, Douglas Adam, Sherborn, MA, UNITED STATES

Lloyd, Clare M., London, UNITED KINGDOM

McCarthy, Sean A., San Diego, CA, UNITED STATES

PA Millennium Pharmaceuticals, Inc. (U.S. corporation)

PI US 2003069196 A1 20030410

AI US 2001-4633 A1 20011204 (10)

RLI Continuation of Ser. No. US 1999-324986, filed on 2 Jun 1999, ABANDONED

Continuation-in-part of Ser. No. US 1998-32337, filed on 27 Feb 1998,

GRANTED, Pat. No. US 6455685 Continuation-in-part of Ser. No. US

1996-609583, filed on 1 Mar 1996, GRANTED, Pat. No. US 6204371

Continuation-in-part of Ser. No. US 1995-487748, filed on 7 Jun 1995,

GRANTED, Pat. No. US 5721351 Continuation-in-part of Ser. No. US

1995-398633, filed on 3 Mar 1995, GRANTED, Pat. No. US 6066322

DT Utility

FS APPLICATION

LREP PENNIE AND EDMONDS, 1155 AVENUE OF THE AMERICAS, NEW YORK, NY, 100362711

CLMN Number of Claims: 21

ECL Exemplary Claim: 1

DRWN 47 Drawing Page(s)

LN.CNT 8502

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to methods and compositions for the treatment and diagnosis of immune disorders, especially T helper lymphocyte-related disorders, and also for the treatment of mast cell-related processes and disorders, ischemic disorders and injuries, including ischemic renal disorders and injuries. For example, genes which are differentially expressed within and among T helper (TH) cells and TH cell subpopulations, which include, but are not limited to TH0, TH1 and TH2 cell subpopulations are identified. Genes are also

identified via the ability of their gene products to interact with gene products involved in the differentiation, maintenance and effector function of such TH cells and TH cell subpopulations. The genes identified can be used diagnostically or as targets for therapeutic intervention. In this regard, the present invention provides methods for the identification and therapeutic use of compounds as treatments of immune disorders, especially TH cell subpopulation-related disorders. Additionally, methods are provided for the diagnostic evaluation and prognosis of TH cell subpopulation-related disorders, for the identification of subjects exhibiting a predisposition to such conditions, for monitoring patients undergoing clinical evaluation for the treatment of such disorders, and for monitoring the efficacy of compounds used in clinical trials. Methods are also provided for the treatment of symptoms associated with mast cell-related processes or disorders and ischemic disorders and injuries using the genes, gene products and ***antibodies*** of the invention.

IN ***Lloyd, Clare M.***, London, UNITED KINGDOM

AB . . . of symptoms associated with mast cell-related processes or disorders and ischemic disorders and injuries using the genes, gene products and ***antibodies*** of the invention.

SUMM . . . TH cells are involved in both humoral and cell-mediated forms of effector immune responses. With respect to the humoral, or ***antibody***, immune response, ***antibodies*** are produced by B lymphocytes through interactions with TH cells. Specifically, extracellular antigens are endocytosed by antigen-presenting cells (APCs), processed, . . . complex (MHC) molecules to CD4.sup.+ class II MHC-restricted TH cells. These TH cells in turn activate B lymphocytes, resulting in ***antibody*** production.

SUMM . . . cells drive humoral immune responses, which are associated with, for example, defenses against certain helminthic parasites, and are involved in ***antibody*** and allergic responses.

SUMM [0026] The present invention also includes the products of such fingerprint, target, and pathway genes, as well as ***antibodies*** to such gene products. Furthermore, the engineering and use of cell- and animal-based models of TH cell subpopulation-related disorders to. . .

SUMM . . . of ischemic disorders or injuries. For example, presented herein are methods of using the 200 gene, its gene product, and ***antibodies*** thereto to treat or regulate ischemic disorders and/or injuries. In particular, the genes or gene products of the invention may. . . to an individual so as to ameliorate the symptoms of the ischemic disorder or injury. Further, compounds, such as specific ***antibodies***, including monoclonal ***antibodies***, which bind specifically to the genes or gene products of the present invention and modulate their expression or activity, may. . .

SUMM . . . of 10 gene product activity, can be particularly amenable to modulation. For example, natural ligands, derivatives of natural ligands and ***antibodies*** which bind to the 10 gene product can be utilized to reduce the number of induced T cells present by. . .

SUMM . . . product, including, for example, soluble fusion proteins such as Ig-tailed fusion proteins, can be particularly useful for this purpose. Additionally, ***antibodies*** directed against one or ore of the extracellular portions of the 10 gene product may either reduce 10 gene product function by, for example, locking ligand binding. Additionally, ***antibodies*** directed against the 10 gene product can, in certain instances, serve to increase the level of 10 gene

product activity.

SUMM . . . IgE immune response, including but not limited to the symptoms which accompany atopic conditions such as allergy and/or asthma.

IgE-type ***antibodies*** are produced by stimulated B cells which require, at least in part, IL-4 produced by the TH2 cell subpopulation.

Therefore, . . .

SUMM . . . one of its gene products is a membrane-bound TH2 cell subpopulation molecule. Accordingly, natural ligands, derivatives of natural ligands and ***antibodies*** which bind to this 103 gene product, can be utilized to reduce the number of TH2 cells present by either. . . for example, soluble fusion proteins such as Ig-tailed fusion proteins, can be particularly useful for this purpose. In certain instances, ***antibodies*** directed against the 103 gene product, such as directed against the extracellular domain of the 103 gene product, can be. . .

SUMM . . . cell subpopulation-restricted, but the Ig superfamily 200 gene product is, additionally, membrane-bound. Therefore, natural ligands, derivatives of natural ligands and ***antibodies*** which bind to the 200 gene product can be utilized to reduce the number of TH1 cells present by either. . .

SUMM [0055] Further, the Example presented in Section 12, below, describes successful use of ***antibodies*** directed against the 103 gene product as well as 103/Ig fusion proteins to ameliorate symptoms of asthma in an accepted. . .

SUMM . . . after injury, particularly after ischemic injury. In particular, the example presented in Section 13, below, demonstrates the successful use of ***antibodies*** which bind to the extracellular domain of the 200 gene product to inhibit repair of ischemic kidney injury. Thus, the. . .

DRWD . . . D10.G4, DAX; TH1: AE7.A, Dorris, D1.1). Clones were either unstimulated (-) or stimulated (+) for 6 hours with plate-bound anti-CD3 ***antibody***. Ten micrograms of total RNA were loaded per lane. The positions of 18s and 28s ribosomal RNA are shown as. . .

DRWD . . . 6, brain; lane 7, heart; lane 8, lung; lane 9, spleen; lane 10, liver. Clones were stimulated with plate-bound anti-CD3 ***antibody*** for 6 hours. 7.5 and 10 micrograms total RNA was used for each cell line and each tissue, respectively. a,. . .

DRWD . . . D10.G4, DAX; TH1: AE7.A, Dorris, D1.1). Clones were either unstimulated (-) or stimulated (+) for 6 hours with plate-bound anti-CD3 ***antibody***. The positions of RNA markers, in kilobases, are shown for reference. The arrow marks the position of 200 gene mRNA.

DRWD . . . (D1.1, Dorris, AE7) cell lines and TH2 (D10.G4, DAX, CDC25) cell lines, either stimulated (+) or unstimulated (-) with anti-CD3 ***antibodies***. 15 micrograms of total RNA were loaded per lane.

Cells were stimulated between 6 and 7 hours with anti-CD3

antibodies, as described, below, in Section 8.1. The Northern blots were hybridized with a probe made from the entire band 54. . .

DRWD . . . (DAX, CDC25) was isolated from either unstimulated cells or from cells which had been stimulated for two hours with anti-CD3

antibodies. 15 micrograms total RNA were loaded per lane. A band 54 DNA probe was used for hybridization.

DRWD . . . surface 103 gene product. The further to the right the peaks are shifted, the greater the staining intensity, and therefore

antibody binding, exhibited by a cell population.

DRWD . . . 103/Ig fusion results in significant decrease in hallmark

symptoms of asthma. FIG. 29A: Animals were treated with the anti-103 3E10 ***antibody*** (listed in the figure as "3E10 MAB"). As a negative control, a set of animals was treated with a non-specific rat Ig ***antibody*** preparation. FIG. 29B: Animals were treated with 103/Ig fusion protein (listed in the figure as "Ig Fus. Prot.") as a negative control, a control set of animals were treated with a non-specific human IgG ***antibody*** preparation.

DRWD . . . a section of untreated mouse kidney tissue; FIG. 31B shows a section of mouse kidney tissue treated with 200 gene ***antibody*** 24 hours prior to, and at 24 hour intervals after the induction of ischemic kidney injury.

DETD [0116] Further, the gene products of such fingerprint, target, and pathway genes are described in Section 5.5, ***antibodies*** to such gene products are described in Section 5.6, as are cell- and animal-based models of TH cell subpopulation differentiation. . .

DETD . . . limited to, pharmacological methods, such as exposure to phorbol esters, calcium ionophores, or lectins (e.g., Concanavalin A), by treatment with ***antibodies*** directed against T-cell receptor epitopes (e.g., anti-CD3 ***antibodies***) or exposure, in the presence of an appropriate antigen presenting cell (APC), to an antigen that the particular TH cells. . .

DETD . . . maintenance, and/or effector function of the subpopulations, using this protein in a manner similar to the well known technique of ***antibody*** probing of .lambda.gt11 libraries.

DETD . . . the pathway and/or differentially expressed genes of the invention also include gene products which are recognized by and bind to ***antibodies*** (polyclonal or monoclonal) directed against the differentially expressed and/or pathway gene products described above; e.g., which are encoded by the. . .

DETD . . . mutant allele. In this manner, gene products made by the putatively mutant tissue can be expressed and screened using standard ***antibody*** screening techniques in conjunction with ***antibodies*** raised against the normal gene product, as described, below, in Section 5.6. (For screening techniques, see, for example, Harlow, E. and Lane, eds., 1988, " ***Antibodies*** : A Laboratory Manual", Cold Spring Harbor Press, Cold Spring Harbor.) In cases where the mutation results in an expressed gene product with altered function (e.g., as a result of a missense mutation), a polyclonal set of ***antibodies*** are likely to cross-react with the mutant gene product. Library clones detected via their reaction with such labeled ***antibodies*** can be purified and subjected to sequence analysis as described in this Section, above.

DETD . . . as many contiguous amino acid residues as necessary to represent an epitope fragment (that is to be recognized by an ***antibody*** directed to the differentially expressed or pathway gene product). For example, such protein fragments or peptides can comprise at least. . .

DETD . . . being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of ***antibodies*** or to screen peptide libraries, for example, vectors which direct the expression of high levels of fusion protein products that. . .

DETD [0229] Alternatively, any fusion protein may be readily purified by utilizing an ***antibody*** specific for the fusion protein being expressed. For example, a system described by Janknecht et al. allows for the ready. . .

DETD [0231] Indirect labeling involves the use of a protein, such as a labeled ***antibody***, which specifically binds to either a differentially expressed or pathway gene product. Such ***antibodies*** include but are not limited to polyclonal, monoclonal, chimeric, single chain, Fab fragments and fragments produced by an Fab expression. . .

DETD 5.6. ***Antibodies*** Specific for Differentially Expressed or Pathway Gene Products

DETD [0241] Described herein are methods for the production of ***antibodies*** capable of specifically recognizing one or more differentially expressed or pathway gene product epitopes. Such ***antibodies*** can include, but are not limited to, polyclonal ***antibodies***, monoclonal ***antibodies*** (mAbs), humanized or chimeric ***antibodies***, single chain ***antibodies***, Fab fragments, F(ab').sub.2 fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) ***antibodies***, and epitope-binding fragments of any of the above. The Ig tails of such ***antibodies*** can be modified to reduce complement activation and Fc binding. (See, for example, European Patent No. 239400 B1, Aug. 3, . .

DETD [0242] Such ***antibodies*** can be used, for example, in the detection of a fingerprint, target, or pathway gene product. in a biological sample, and can be used as part of diagnostic techniques. Alternatively, such ***antibodies*** can be utilized as part of an immune disorder treatment method, as described, below, in Section 5.9. For example, the ***antibodies*** can be used to modulate target gene activity, can be used to modulate TH cell subpopulation differentiation, maintenance and/or effector function, or, in the case of ***antibodies*** directed to cell surface epitopes, can be used to isolate a TH cell subpopulation of interest, for either depletion or. . .

DETD [0243] Such ***antibodies*** can also be utilized as part of a method for treatment of an ischemic disorder or injury, as described in Section 5.10.3.1, below. For example, the ***antibodies*** can be used to block or inhibit activity of one or more of the gene products of the invention, thereby. . .

DETD [0244] For the production of ***antibodies*** to a differentially expressed or pathway gene, various host animals can be immunized by injection with a differentially expressed or. . .

DETD [0245] Polyclonal ***antibodies*** are heterogeneous populations of ***antibody*** molecules derived from the sera of animals immunized with an antigen, such as target gene product, or an antigenic functional derivative thereof. For the production of polyclonal ***antibodies***, host animals such as those described above, can be immunized by injection with differentially expressed or pathway gene product supplemented. . .

DETD [0246] Monoclonal ***antibodies***, which are homogeneous populations of ***antibodies*** to a particular antigen, can be obtained by any technique which provides for the production of ***antibody*** molecules by continuous cell lines in culture. These include, but are not limited to the hybridoma technique of Kohler and. . . 4:72; Cole et al., 1983, Proc. Natl. Acad. Sci. USA 80:2026-2030), and the EBV-hybridoma technique (Cole et al., 1985, Monoclonal ***Antibodies*** And Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). Such ***antibodies*** can be of any immunoglobulin class including

IgG, IgM, IgE, IgA, IgD and any subclass thereof. The hybridoma producing the. . .

DETD [0247] In addition, techniques developed for the production of "chimeric ***antibodies***" (Morrison et al., 1984, Proc. Natl. Acad. Sci., 81:6851-6855; Neuberger et al., 1984, Nature, 312:604-608; Takeda et al., 1985, Nature, 314:452-454; U.S. Pat. No. 4,816,567) by splicing the genes from a mouse ***antibody*** molecule of appropriate antigen specificity together with genes from a human ***antibody*** molecule of appropriate biological activity can be used. A chimeric ***antibody*** is a molecule in which different portions are derived from different animal species, such as those having a variable region.

DETD [0248] Alternatively, techniques described for the production of single chain ***antibodies*** (U.S. Pat. No. 4,946,778; Bird, 1988, Science 242:423-426; Huston et al., 1988, Proc. Natl. Acad. Sci. USA 85:5879-5883; and Ward et al., 1989, Nature 334:544-546) and for making humanized monoclonal ***antibodies*** (U.S. Pat. No. 5,225,539, which is incorporated herein by reference in its entirety) can be utilized to produce anti-differentially expressed or anti-pathway gene product ***antibodies***.

DETD [0249] ***Antibody*** fragments which recognize specific epitopes can be generated by known techniques. For example, such fragments include but are not limited to: the F(ab').sub.2 fragments which can be produced by pepsin digestion of the ***antibody*** molecule and the Fab fragments which can be generated by reducing the disulfide bridges of the F(ab').sub.2 fragments. Alternatively, Fab. . .

DETD [0250] ***Antibodies*** to the differentially expressed or pathway gene products can, in turn, be utilized to generate anti-idiotypic ***antibodies*** that "mimic" such gene products, using techniques well known to those skilled in the art. (See, e.g., Greenspan & Bona, . . Nissinoff, 1991, J. Immunol. 147(8):2429-2438). For example, in the case of receptor-type molecules (e.g., 10, 103 and 200 gene products) ***antibodies*** which bind to the ECD and competitively inhibit the binding of ligand to the receptor can be used to generate.

DETD [0251] Production of ***antibodies*** directed against the extracellular domain of the 103 gene product are described in Section 12, below. Also, production of ***antibodies*** directed against the extracellular domain of the 200 gene product are described in Section 13, below.

DETD . . . from the animal, in situ hybridization analysis, and RT-PCR. Samples of target gene-expressing tissue, can also be evaluated immunocytochemically using ***antibodies*** specific for the target gene transgene gene product of interest.

DETD [0265] The target gene transgenic animals that express target gene mRNA or target gene transgene peptide (detected immunocytochemically, using ***antibodies*** directed against target gene product epitopes) at easily detectable levels can then be further evaluated to identify those animals which. . .

DETD . . . limited to members of random or partially degenerate, directed phosphopeptide libraries; see, e.g., Songyang, Z. et al., 1993, Cell 72:767-778), ***antibodies*** (including, but not limited to polyclonal, monoclonal, humanized, anti-idiotypic, chimeric or single chain ***antibodies***, and FAb, F(ab').sub.2 and FAb expression library fragments, and epitope-binding fragments thereof), and small

organic or inorganic molecules. In the . . . bind to the ECD and either mimic the activity triggered by the natural ligand (i.e., agonists); as well as peptides, ***antibodies*** or fragments thereof, and other organic compounds that mimic the ECD (or a portion thereof) and bind to a "neutralize". . .

DETD . . . can be accomplished by simply coating the solid surface with a solution of the protein and drying. Alternatively, an immobilized ***antibody***, preferably a monoclonal ***antibody***, specific for the protein to be immobilized can be used to anchor the protein to the solid surface. The surfaces. . .

DETD . . . is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; e.g., using a labeled ***antibody*** specific for the previously nonimmobilized component (the ***antibody***, in turn, can be directly labeled or indirectly labeled with a labeled anti-Ig ***antibody***).

DETD . . . be conducted in a liquid phase, the reaction products separated from unreacted components, and complexes detected; e.g., using an immobilized ***antibody*** specific for target gene product or the test compound to anchor any complexes formed in solution, and a labeled ***antibody*** specific for the other component of the possible complex to detect anchored complexes.

DETD . . . target gene protein, especially mutant target gene proteins. Such compounds can include, but are not limited to molecules such as ***antibodies***, peptides, and the like, as described, for example, in Section 5.8.1. above.

DETD . . . coating the solid surface with a solution of the target gene product or binding partner and drying. Alternatively, an immobilized ***antibody*** specific for the species to be anchored can be used to anchor the species to the solid surface. The surfaces. . .

DETD . . . is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; e.g., using a labeled ***antibody*** specific for the initially non-immobilized species (the ***antibody***, in turn, can be directly labeled or indirectly labeled with a labeled anti-Ig ***antibody***). Depending upon the order of addition of reaction components, test compounds which inhibit complex formation or which disrupt preformed complexes. . .

DETD . . . or absence of the test compound, the reaction products separated from unreacted components, and complexes detected; e.g., using an immobilized ***antibody*** specific for one of the binding components to anchor any complexes formed in solution, and a labeled ***antibody*** specific for the other partner to detect anchored complexes. Again, depending upon the order of addition of reactants to the. . .

DETD . . . the resulting fusion protein. The interactive cellular or extracellular binding partner can be purified and used to raise a monoclonal ***antibody***, using methods routinely practiced in the art and described above, in Section 5.6. This ***antibody*** can be labeled with the radioactive isotope ¹²⁵I, for example, by methods routinely practiced in the art. In a heterogeneous. . . binding to occur. At the end of the reaction period, unbound material can be washed away, and the labeled monoclonal ***antibody*** can be added to the system and allowed to bind to the complexed components. The interaction between the target gene. . .

DETD . . . away. Again the extent of inhibition of the target gene product/binding partner interaction can be detected by adding the

labeled ***antibody*** and measuring the radioactivity associated with the beads.

DETD . . . describes the successful utilization of a 103 gene product/Ig fusion protein, as well as the successful use of a monoclonal ***antibody*** directed against the extracellular domain of the 103 gene product to ameliorate symptoms of asthma in an accepted animal model. . .

DETD . . . limited to members of random or partially degenerate, directed phosphopeptide libraries; see, e.g., Songyang, Z. et al., 1993, Cell 72:767-778), ***antibodies*** (including, but not limited to polyclonal, monoclonal, humanized, anti-idiotypic, chimeric or single chain ***antibodies***, and FAb, F(ab').sub.2 and FAb expression library fragments, and epitope-binding fragments thereof), and small organic or inorganic molecules.

DETD . . . soluble Ig-tailed fusion proteins which may be produced by utilizing techniques such as those described, above, in Section 5.5. Additionally, ***antibodies*** directed against the extracellular portion of the 10 gene product may reduce 10 gene product function by, for example, blocking. . .

DETD . . . for example, peptides representing soluble extracellular portions of target gene product transmembrane receptors), phosphopeptides, small organic or inorganic molecules, or ***antibodies*** (including, for example, polyclonal, monoclonal, humanized, anti-idiotypic, chimeric or single chain ***antibodies***, and FAb, F(ab').sub.2 and FAb expression library fragments, and epitope-binding fragments thereof). In one embodiment, for example, ***antibodies*** directed against a 103 gene product, preferably an extracellular or extracellular portion of a 103 gene product, can be utilized.. . .

DETD . . . IgE immune responses, including, but not limited to the symptoms which accompany atopic conditions such as allergy and/or asthma. IgE-type ***antibodies*** are produced by stimulated B cells which require, at least in part, IL-4 produced by the TH2 cell subpopulation. Therefore,. . .

DETD [0351] For example, natural ligands, derivatives of natural ligands and ***antibodies*** which bind to the 103 gene product can be utilized to reduce the number of TH2 cells present by either. . .

DETD [0357] For example, natural ligands, derivatives of natural ligands and ***antibodies*** which bind to the 200 gene product can be utilized to reduce the number of TH1 cells present by either. . .

DETD . . . and/or analogs thereof, of the gene 200 product, including, for example, soluble fusion proteins such as Ig-tailed fusion proteins or ***antibodies***. (For a discussion of the production of Ig-tailed fusion proteins see, for example, U.S. Pat. No. 5,116,964.)

DETD . . . these 200 gene product domains or mutants fused to another polypeptide (e.g., an IgFc polypeptide) can be utilized. Alternatively, anti-idiotypic ***antibodies*** or Fab fragments of antiidiotypic ***antibodies*** that mimic the 200 gene product ECD and neutralize 200 gene product ligand can be used. Such 200 gene product peptides, proteins, fusion proteins, anti-idiotypic ***antibodies*** or Fabs are administered to a subject in amounts sufficient to neutralize the gene product and thereby effectuate an amelioration. . .

DETD . . . into the tissue site, or modified antisense molecules, designed to target the desired cells (e.g., antisense linked to peptides or ***antibodies*** that specifically bind receptors or antigens

expressed on the target cell surface) can be administered systemically.

DETD . . . also include peptides representing soluble extracellular portions of target gene product transmembrane proteins, phosphopeptides, small organic or inorganic molecules, or ***antibodies*** (including, for example, polyclonal, monoclonal, humanized, anti-idiotypic, chimeric or single chain ***antibodies***, and FAb, F(ab.sup.1).sub.2 and FAb expression library fragments, and epitope-binding fragments thereof).

DETD 5.10.3.1. ***Antibody*** Techniques

DETD [0402] ***Antibodies*** exhibiting modulatory capability can be utilized to ameliorate immune disorders such as TH cell subpopulation-related disorders, or to treat ischemic disorders and injuries. Depending on the specific ***antibody***, the modulatory effect can be negative and can, therefore, be utilized as part of the techniques described, above, in Section. . .

DETD [0403] An ***antibody*** having negative modulatory capability refers to an ***antibody*** which specifically binds to and interferes with the action of a protein. In the case of an extracellular receptor, for example, such an ***antibody*** would specifically bind the extracellular domain of the receptor in a manner which does not activate the receptor but which disrupts the ability of the receptor to bind its natural ligand. For example, ***antibodies*** directed against the extracellular domains of genes 103 or 200 can function as such negative modulators. Additionally, ***antibodies*** directed against one or more of the 10 gene product extracellular domains can function in a negative modulatory manner. Such ***antibodies*** can be generated using standard techniques described in Section 5.6, above, against full length wild type or mutant proteins, or against peptides corresponding to portions of the proteins. The ***antibodies*** include but are not limited to polyclonal, monoclonal, FAb fragments, single chain ***antibodies***, chimeric ***antibodies***, and the like.

DETD [0404] An ***antibody*** having positive modulatory capability refers to an ***antibody*** which specifically binds to a protein and, by binding, serves to, either directly or indirectly, activate the function of the protein which it recognizes. For example, an ***antibody*** can bind to the extracellular portion of a transmembrane protein in a manner which causes the transmembrane protein to function as though its endogenous ligand was binding, thus activating, for example, a signal transduction pathway.

antibodies can be generated using standard techniques described in Section 5.6, above, against full length wild type or mutant proteins, or against peptides corresponding to portions of the proteins. The ***antibodies*** include but are not limited to polyclonal, monoclonal, FAb fragments, single chain ***antibodies***, chimeric ***antibodies***, and the like.

DETD [0405] In instances where the protein, such as a target gene protein, to which the ***antibody*** is directed is intracellular and whole ***antibodies*** are used, internalizing ***antibodies*** can be preferred. However, lipofectin or liposomes can be used to deliver the ***antibody*** or a fragment of the Fab region which binds to the gene product epitope into cells. Where fragments of the ***antibody*** are used, the smallest inhibitory fragment which binds to the protein's binding domain is preferred. For example, peptides having an amino acid sequence corresponding to the domain of the variable region of the

antibody that binds to the protein can be used. Such peptides can be synthesized chemically or produced via recombinant DNA technology. . . methods well known in the art (e.g., see Creighton, 1983, supra; and Sambrook et al., 1989, above). Alternatively, single chain ***antibodies***, such as neutralizing ***antibodies***, which bind to intracellular epitopes can also be administered. Such single chain ***antibodies*** can be administered, for example, by expressing nucleotide sequences encoding single-chain ***antibodies*** within the target cell population by utilizing, for example, techniques such as those described in Marasco et al. (Marasco, W. . .

DETD [0406] In instances where the protein to which the ***antibody*** is directed is extracellular, or is a transmembrane protein, any of the administration techniques described, below in Section 5.11 which are appropriate for peptide administration can be utilized to effectively administer the ***antibodies*** to their site of action.

DETD . . . of the separation is to increase or augment the number of cells belonging to a specific TH cell subpopulation, the ***antibodies*** used can also be specific to surface markers present on undifferentiated or partially undifferentiated TH cells. After separation, and purification. . .

DETD . . . such as TH1 or TH2 cells, can be removed, and thus selectively separated and purified, by various methods which utilize ***antibodies*** which bind specific markers present on the T cell population of interest, while absent on other cells within the starting. . . a fluorescence activated cell sorter (FACS) and specific fluorochromes, biotin-avidin or biotin-streptavidin separations using biotin conjugated to cell surface marker-specific ***antibodies*** and avidin or streptavidin bound to a solid support such as affinity column matrix or plastic surfaces or magnetic separations using ***antibody***-coated magnetic beads.

DETD [0413] Separation via ***antibodies*** for specific markers can be by negative or positive selection procedures. In negative separation, ***antibodies*** are used which are specific for markers present on undesired cells. For example, in the case of a TH1 cell subpopulation-related disorder wherein it would be desirable to deplete the number of TH1 cells, such ***antibodies*** could be directed to the extracellular domain of the 200 gene product. Alternatively, in the case of TH2 cell subpopulation-related disorders wherein it would be desirable to deplete the number of TH1 cells, such ***antibodies*** could be directed to the extracellular domain of the 103 gene product. Cells bound by an ***antibody*** to such a cell surface marker can be removed or lysed and the remaining desired mixture retained.

DETD [0414] In positive separation, ***antibodies*** specific for markers present on the desired cells of interest. For example, in the case of a TH1 cell subpopulation-related disorder wherein it would be desirable to increase the number of TH1 cells, such ***antibodies*** could be directed to the extracellular domain of the 200 gene product. Alternatively, in the case of TH2 cell subpopulation-related disorders wherein it would be desirable to increase the number of TH1 cells, such ***antibodies*** could be directed to the extracellular domain of the 103 gene product. Cells bound by the ***antibody*** are separated and retained. It will be understood that positive and negative separations can be used substantially simultaneously or in. . .

DETD [0415] A common technique for ***antibody*** based separation is the use of flow cytometry such as by a fluorescence activated cell sorter

(FACS). Typically, separation by flow cytometry is performed as follows. The suspended mixture of cells are centrifuged and resuspended in media.

Antibodies which are conjugated to fluorochrome are added to allow the binding of the ***antibodies*** to specific cell surface markers. The cell mixture is then washed by one or more centrifugation and resuspension steps. The. . .

DETD . . . such method is biotin-avidin based separation by affinity chromatography. Typically, such a technique is performed by incubating cells with biotin-coupled ***antibodies*** to specific markers, such as, for example, the transmembrane protein encoded by the 103 gene described herein, followed by passage through an avidin column. Biotin-***antibody***-cell complexes bind to the column via the biotin-avidin interaction, while other cells pass through the column. The specificity of the. . .

DETD . . . proliferation of the purified TH cell subpopulation. Such an expansion agent can, for example, be any appropriate cytokine, antigen, or ***antibody***. In the case of TH2 cells, for example, the expansion agent can be IL-4, while for TH1 cells, the expansion. . .

DETD [0425] Targeting moieties can include, but are not limited to, ***antibodies*** directed to cell surface markers found specifically on the TH cell subpopulation being targeted, or, alternatively, to ligands, such as. . .

DETD [0426] In the case of TH2 cells, for example, such a targeting moiety can represent an ***antibody*** directed against the extracellular portion of the 103 gene product described herein, or can, alternatively, represent a ligand specific for. . . this receptor-type TH2 specific molecule. In the case of TH1 cells, for example, such a targeting moiety can represent an ***antibody*** directed against the extracellular portion of the 200 gene product described herein, or can, alternatively, represent a ligand specific for. . .

DETD [0431] As defined herein, a therapeutically effective amount of ***antibody***, protein, or polypeptide (i.e., an effective dose or effective dosage) ranges from about 0.001 to 30 mg/kg of body weight,. . .

DETD . . . subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a protein, polypeptide or ***antibody*** can include a single treatment or, preferably, can include a series of treatments. In a preferred example, a subject is treated with ***antibody***, protein, or polypeptide in the range of between about 0.1 to 20 mg/kg body weight one time per week for. . . even more preferably for about 4, 5, or 6 weeks. It will also be appreciated that the effective dosage of ***antibody***, protein or polypeptide used for treatment may increase or decrease over the course of a particular treatment. Changes in dosage. . .

DETD 5.11.3. Pharmaceutical Preparation and Administration of
Antibodies

DETD [0444] ***Antibodies*** which specifically bind to target gene products of the invention and thereby modulate their activity can also be administered to. . . to treat or ameliorate ischemic disorders or injuries. For example, Section 13, below, demonstrates the use of anti-200 gene product ***antibodies*** to block recovery of kidney tissue from ischemia reperfusion injury.

DETD [0445] ***Antibodies*** of the invention are administered by any suitable means, including those described in Section 5.12.2, above. In addition, ***antibody*** to a target gene product of the invention

is suitably administered by pulse infusion, particularly with declining doses of the ***antibody***. Preferably, the dosing is administered by injections, most preferably by intravenous or subcutaneous injections, depending in part on whether the. . .

DETD [0446] The appropriate dosage of ***antibody*** will depend on the type of disease to be treated, the severity and course of the disease, whether the ***antibody*** is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the ***antibody***, and the discretion of the attending physician. The ***antibody*** may suitably administered to the patient at one time or, more preferably, over a series of treatments.

DETD [0447] As a general proposition, the initial pharmaceutically effective amount of ***antibody*** administered parenterally will be in the range of about 0.1 to 20 mg/kg of patient body weight per day, with. .

DETD [0453] Such methods can, for example, utilize reagents such as the fingerprint gene nucleotide sequences described in Sections 5.1, and ***antibodies*** directed against differentially expressed and pathway gene peptides, as described, above, in Sections 5.5 (peptides) and 5.6 (***antibodies***). Specifically, such reagents can be used, for example, for: 1) the detection of the presence of target gene expression, target. . .

DETD . . . performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one specific fingerprint gene nucleic acid or anti-fingerprint gene ***antibody*** reagent described herein, which can be conveniently used, e.g., in clinical settings, to diagnose patients exhibiting TH1- or TH2-related abnormalities.

DETD . . . stimulating the cells prior to contacting the cells with the compound. Among the methods for stimulation are stimulation via anti-CD3 ***antibody*** stimulation.

DETD [0478] ***Antibodies*** directed against wild type or mutant fingerprint gene peptides, which are discussed, above, in Section 5.6, can also be used. . .

DETD . . . employed herein can, for example, be such as those described in Harlow and Lane (Harlow, E. and Lane, D., 1988, " ***Antibodies*** : A Laboratory Manual", Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.), which is incorporated herein by reference in its. . .

DETD . . . molecules can involve, for example, immunoassays wherein fingerprint gene peptides are detected by their interaction with an anti-fingerprint gene product-specific ***antibody***.

DETD [0481] For example, ***antibodies***, or fragments of ***antibodies***, such as those described, above, in Section 5.6, useful in the present invention can be used to quantitatively or qualitatively. . . wild type or mutant fingerprint gene peptides. This can be accomplished, for example, by immunofluorescence techniques employing a fluorescently labeled ***antibody*** (see below, this Section,) coupled with light microscopic, flow cytometric, or fluorimetric detection. Such techniques are especially preferred if the. . .

DETD [0482] The ***antibodies*** (or fragments thereof) useful in the present invention can, additionally, be employed histologically, as in immunofluorescence or immunoelectron microscopy, for. . . peptides. In situ detection can be accomplished by removing a histological specimen from a patient, and applying thereto a labeled ***antibody***

of the present invention. The ***antibody*** (or fragment) is preferably applied by overlaying the labeled ***antibody*** (or fragment) onto a biological sample. Through the use of such a procedure, it is possible to determine not only. . .

DETD . . . extract, freshly harvested cells, or cells which have been incubated in tissue culture, in the presence of a detectably labeled ***antibody*** capable of identifying fingerprint gene peptides, and detecting the bound ***antibody*** by any of a number of techniques well-known in the art.

DETD . . . soluble proteins. The support can then be washed with suitable buffers followed by treatment with the detectably labeled fingerprint gene-specific ***antibody***. The solid phase support can then be washed with the buffer a second time to remove unbound ***antibody***. The amount of bound label on solid support can then be detected by conventional means.

DETD [0485] By "solid phase support or carrier" is intended any support capable of binding an antigen or an ***antibody***. Well-known supports or carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, gabbros, and magnetite.. . have virtually any possible structural configuration so long as the coupled molecule is capable of binding to an antigen or ***antibody***. Thus, the support configuration can be spherical, as in a bead, or cylindrical, as in the inside surface of a . . . strip, etc. Preferred supports include polystyrene beads. Those skilled in the art will know many other suitable carriers for binding ***antibody*** or antigen, or will be able to ascertain the same by use of routine experimentation.

DETD [0486] The binding activity of a given lot of anti-wild type or mutant fingerprint gene product ***antibody*** can be determined according to well known methods. Those skilled in the art will be able to determine operative and. . .

DETD [0487] One of the ways in which the fingerprint gene peptide-specific ***antibody*** can be detectably labeled is by linking the same to an enzyme and use in an enzyme immunoassay (EIA) (Voller,. . . Raton, Fla.; Ishikawa, E. et al., (eds.), 1981, ENZYME IMMUNOASSAY, Kogaku Shoin, Tokyo). The enzyme which is bound to the ***antibody*** will react with an appropriate substrate, preferably a chromogenic substrate, in such a manner as to produce a chemical moiety. . . be detected, for example, by spectrophotometric, fluorimetric or by visual means. Enzymes which can be used to detectably label the ***antibody*** include, but are not limited to, malate dehydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, yeast alcohol dehydrogenase, alpha-glycerophosphate, dehydrogenase, triose phosphate isomerase,. .

DETD [0488] Detection can also be accomplished using any of a variety of other immunoassays. For example, by radioactively labeling the ***antibodies*** or ***antibody*** fragments, it is possible to detect fingerprint gene wild type or mutant peptides through the use of a radioimmunoassay (RIA). . .

DETD [0489] It is also possible to label the ***antibody*** with a fluorescent compound. When the fluorescently labeled ***antibody*** is exposed to light of the proper wavelength, its presence can then be detected due to fluorescence. Among the most. . .

DETD [0490] The ***antibody*** can also be detectably labeled using fluorescence emitting metals such as .sup.152Eu, or others of the

lanthamide series. These metals can be attached to the ***antibody*** using such metal chelating groups as diethylenetriaminepentacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA).

DETD [0491] The ***antibody*** also can be detectably labeled by coupling it to a chemiluminescent compound. The presence of the chemiluminescent-tagged ***antibody*** is then determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of. . .

DETD [0492] Likewise, a bioluminescent compound can be used to label the ***antibody*** of the present invention. Bioluminescence is a type of chemiluminescence found in biological systems in, which a catalytic protein increases. . .

DETD . . . samples were isolated from either unstimulated cells or from cells which had been stimulated for 6 hours with plate-bound anti-CD3 ***antibody***. The samples were probed with band 103 sequences, as shown in FIG. 6. While 103 gene RNA is present in. . .

DETD . . . E. et al., John Wiley & Sons, NY, pp 3.12.4-3.12.6).

Specifically, prior to plating, the flasks were coated with anti-CD3-epsilon. ***antibodies*** (hybridoma supernatant from the 145-C11 hybridoma; Parmingen, Inc., San Diego Calif.). For coating, ***antibodies*** were resuspended in PBS at 1-2 .mu.g/ml at a volume sufficient to coat the bottom of the flasks. Coating solution. . .

DETD [0551] After incubation, the ***antibody*** coating solution was removed by aspiration and cells were immediately added. Flasks were placed in a 37.degree. C. incubator for. . .

DETD . . . samples were isolated from either unstimulated cells (-) or cells which had been stimulated for 6 hours with plate-bound anti-CD3 ***antibody*** (+). The samples were probed with 200 gene sequences, and, as shown in FIG. 18, RNA from both stimulated and. . .

DETD . . . blot data depicted in FIG. 19 additionally illustrates 54 gene expression within cell clones either stimulated or unstimulated with anti-CD3 ***antibodies***, and demonstrates that 54 gene expression goes down within stimulated TH1 cells.

DETD . . . Specifically, RNA was isolated from unstimulated AE7 clones as well as from AE7 clones which had been stimulated with anti-CD3 ***antibodies*** for varying lengths of time, as noted in FIG. 20. As illustrated in FIG. 20, 54 gene expression decreased slightly. . .

DETD . . . herein presents in vivo data demonstrating that the 103 gene product regulates TH2 effector cell responses. In particular, a monoclonal ***antibody*** (3E10 mAb) has been generated against the 103 gene product, and its effect in an adoptive transfer model of TH1.

DETD [0646] Rat monoclonal ***antibodies*** (MAbs), including the 3E10 MAb, were generated against the extracellular domain of the mouse 103 gene product. A DNA sequence. . .

DETD . . . culture supernatant (or 1 .mu.g purified 3E10 protein) was applied 1.times.10.sup.6 cells. After rinsing, cells were contacted with goat anti-rat ***antibody*** conjugated with PE (R-phycoerythrin) fluorescent dye. After a final rinse, cell analysis was carried out on a FACS Vantage (Becton. . .

DETD [0682] To further investigate the expression and role of the 103 gene product in TH cells, a monoclonal ***antibody*** (3E10 mAb) directed against the extracellular domain of the 103 gene product was prepared and characterized.

DETD . . . corroborating the results presented in the Example of Section

7, above. In addition, the data demonstrate the feasibility of using ***antibodies*** to separate TH2 subpopulation cells away from other cell types, thereby modulating a TH cell subpopulation by changing the number. . .

DETD . . . vivo data that the 200 gene product is involved in the recovery from kidney ischemia injury. In particular, a monoclonal ***antibody*** (96.3.8H7 mAb) generated against the extracellular domain of the murine 200 gene product, and its effect in a surgical model. . .

DETD [0703] Rat monoclonal ***antibodies*** (mAbs) were generated against the extracellular domain of the mouse 200 gene product. Recombinant murine 200 gene IgG1 (m200Ig) fusion. . .

DETD . . . For 200 gene product blockage experiments, mice were pretreated 24 hours before surgery with 100 .mu.g/mouse of rat anti-200 monoclonal ***antibody*** (200 mAb). In control experiments, mice were administered equivalent dosages of rat Ig (RtIg) ***antibody*** .

DETD [0708] Mice were then given ***antibody*** at 24 hour intervals following surgical recovery (4 hours post anesthetic).

DETD [0713] A monoclonal ***antibody*** (96.3.8H7 mAb) directed against the extracellular domain of the 200 gene product was prepared and characterized, and this ***antibody*** was administered to mice 24 hours before, and at 24 hour intervals following ischemic kidney injury. Control animals were administered. . .

DETD . . . high levels of 103 gene expression in a human mast cell line. The example also describes the production of monoclonal ***antibodies*** which are specific for the human, but not mouse, 103 gene product. FAC staining of the human mast cell line demonstrated binding of these monoclonal ***antibodies***, confirming that the 103 gene product is, indeed, expressed in mast cells.

DETD [0719] Monoclonal ***antibodies*** (mAbs; see below) were generated against the extracellular domain (amino acid residues 18-323) of a human 103 gene product (with. . .

DETD . . . protein using fluorescence activated cells sorting (FACS) according to standard methods described in Section 12.1, above using anti-mouse IgFITC secondary ***antibodies*** .

DETD . . . in a human mast cell line. Expression of the 103 gene product in this cell line was verified using monoclonal ***antibodies*** raised against an Fc fusion protein of the human 103 gene product, as described in Section 14.1, above.

DETD [0732] FACS staining of the human mast cell line, with the 21 monoclonal ***antibodies*** showed staining with 15 of the 21 ***antibodies*** compared to isotype controls. Five of these 15 ***antibodies***, identified as 1B4, 2O3, 3F7, 3H18, and 10F7, were selected for further analysis. FAC staining with these ***antibodies*** was demonstrated to be specifically blocked with an excess of human 103-Fc fusion protein, however, staining was not blocked with. . .

DETD . . . is expressed in a human mast cell line. Accordingly, the 103 gene, its gene product, and compositions derived therefrom (e.g., ***antibodies*** and other compounds which bind to and/or modulate the expression or activity of the 103 gene or its gene product). . .

CLM What is claimed is:

. . . method for ameliorating a symptom of an ischemic disorder or injury in a mammal, comprising administering to the mammal an ***antibody*** directed against a 200 gene product in an amount effective to ameliorate the symptom of the disorder.

17. The method of claim 3 wherein said administering of the ***antibody*** is parenteral, subcutaneous, intraperitoneal, intrapulmonary, intranasal, or intralesional.

18. The method of claim 17, wherein the intralesional administration comprises perfusing or contacting a graft or organ with the ***antibody*** before transplant.

19. The method of claim 3, wherein the amount of the ***antibody*** administered is from about 1 .mu.g/kg to about 100 mg/kg.

20. The method of claim 19, wherein the amount of the ***antibody*** administered is from about 1 .mu.g/kg to about 15 mg/kg.

21. The method of claim 20, wherein the amount of the ***antibody*** administered is from about 0.1 mg/kg to about 2.0 mg/kg.

=> e mccarthy sean/au

```
E1      1  MCCARTHY SCOTT/AU
E2      1  MCCARTHY SCOTT J/AU
E3     12 --> MCCARTHY SEAN/AU
E4     63  MCCARTHY SEAN A/AU
E5     11  MCCARTHY SEAN ANTHONY/AU
E6      2  MCCARTHY SEAN J/AU
E7     11  MCCARTHY SEAN M/AU
E8      6  MCCARTHY SEAN P/AU
E9     15  MCCARTHY SEAN T/AU
E10     1  MCCARTHY SEAN THOMAS/AU
E11     1  MCCARTHY SHANLEY K/AU
E12     12  MCCARTHY SHARON/AU
```

=> e4-e5 and antibod?

E4-E5 IS NOT A RECOGNIZED COMMAND

The previous command name entered was not recognized by the system.

For a list of commands available to you in the current file, enter

"HELP COMMANDS" at an arrow prompt (=>).

=> s e4-e5 and antibod?

```
L7     43 ("MCCARTHY SEAN A"/AU OR "MCCARTHY SEAN ANTHONY"/AU) AND ANTIBOD
?
```

=> dup rem l7

PROCESSING COMPLETED FOR L7

```
L8     33 DUP REM L7 (10 DUPLICATES REMOVED)
```

=> s l8 and ischem?

```
L9      9 L8 AND ISCHEM?
```

=> d bib ab kwic 1-

YOU HAVE REQUESTED DATA FROM 9 ANSWERS - CONTINUE? Y/(N):y

```
L9  ANSWER 1 OF 9  CAPLUS  COPYRIGHT 2003 ACS on STN
```

AN 2000:861842 CAPLUS

DN 134:28454

TI T helper cell-expressed polynucleotide and polypeptide compositions and methods for the treatment and diagnosis of immune disorders

IN Levinson, Douglas A.; Lloyd, Clare M.; ***McCarthy, Sean A.***

PA Millennium Pharmaceuticals, Inc., USA

SO PCT Int. Appl., 311 pp.

CODEN: PIXXD2

DT Patent

LA English

FAN.CNT 5

PATENT NO. KIND DATE APPLICATION NO. DATE

PI WO 2000073498 A1 20001207 WO 2000-US14986 20000531

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRAI US 1999-324986 A 19990602

AB The present invention relates to methods and compns. for the treatment and diagnosis of immune disorders, esp. T helper lymphocyte-related disorders, and also for the treatment of mast cell-related processes and disorders, ***ischemic*** disorders and injuries, including ***ischemic*** renal disorders and injuries. For example, genes which are differentially expressed within and among T helper (TH) cells and TH cell subpopulations, which include, but are not limited to TH0, TH1 and TH2 cell subpopulations are identified. Genes are also identified via the ability of their gene products to interact with gene products involved in the differentiation, maintenance and effector function of such TH cells and TH cell subpopulations. The genes identified can be used diagnostically or as targets for therapeutic intervention. In this regard, the present invention provides methods for the identification and therapeutic use of compds. as treatments of immune disorders, esp. TH cell subpopulation-related disorders. Addnl., methods are provided for the diagnostic evaluation and prognosis of TH cell subpopulation-related disorders, for the identification of subjects exhibiting a predisposition to such conditions, for monitoring patients undergoing clin. evaluation for the treatment of such disorders, and for monitoring the efficacy of compds. used in clin. trials. Methods are also provided for the treatment of symptoms assocd. with mast cell-related processes or disorders and ***ischemic*** disorders and injuries using the genes, gene products and ***antibodies*** of the invention.

RE.CNT 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

IN Levinson, Douglas A.; Lloyd, Clare M.; ***McCarthy, Sean A.***

AB The present invention relates to methods and compns. for the treatment and diagnosis of immune disorders, esp. T helper lymphocyte-related disorders, and also for the treatment of mast cell-related processes and disorders, ***ischemic*** disorders and injuries, including ***ischemic*** renal disorders and injuries. For example, genes which are differentially

expressed within and among T helper (TH) cells and TH cell subpopulations, which include, but are not limited to TH0, TH1 and TH2 cell subpopulations are identified. Genes are also identified via the ability of their gene products to interact with gene products involved in the differentiation, maintenance and effector function of such TH cells and TH cell subpopulations. The genes identified can be used diagnostically or as targets for therapeutic intervention. In this regard, the present invention provides methods for the identification and therapeutic use of compds. as treatments of immune disorders, esp. TH cell subpopulation-related disorders. Addnl., methods are provided for the diagnostic evaluation and prognosis of TH cell subpopulation-related disorders, for the identification of subjects exhibiting a predisposition to such conditions, for monitoring patients undergoing clin. evaluation for the treatment of such disorders, and for monitoring the efficacy of compds. used in clin. trials. Methods are also provided for the treatment of symptoms assocd. with mast cell-related processes or disorders and ***ischemic*** disorders and injuries using the genes, gene products and ***antibodies*** of the invention.

IT ***Ischemia***

Mast cell

Protein sequences

cDNA sequences

(T helper cell-expressed polynucleotide and polypeptide compns. and methods for the treatment and diagnosis of immune disorders)

IT Heart, disease

Kidney, disease

(***ischemia*** ; T helper cell-expressed polynucleotide and polypeptide compns. and methods for the treatment and diagnosis of immune disorders)

L9 ANSWER 2 OF 9 USPATFULL on STN

AN 2003:257733 USPATFULL

TI Novel human Delta3 compositions and therapeutic and diagnostic uses therefor

IN ***McCarthy, Sean A.*** , San Diego, CA, UNITED STATES

Gearing, David P., East Doncaster, AUSTRALIA

PA Millennium Pharmaceuticals, Inc. (U.S. corporation)

PI US 2003180784 A1 20030925

AI US 2003-417719 A1 20030417 (10)

RLI Continuation of Ser. No. US 2000-568218, filed on 9 May 2000, PENDING

Continuation-in-part of Ser. No. US 1997-872855, filed on 11 Jun 1997,

GRANTED, Pat. No. US 6121045 Continuation-in-part of Ser. No. US

1997-832633, filed on 4 Apr 1997, ABANDONED

DT Utility

FS APPLICATION

LREP MILLENNIUM PHARMACEUTICALS, INC., 75 Sidney Street, Cambridge, MA, 02139

CLMN Number of Claims: 35

ECL Exemplary Claim: 1

DRWN 10 Drawing Page(s)

LN.CNT 11165

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention provides nucleic acids encoding Delta3 proteins. Also provided are derivatives of Delta3 nucleic acids, polypeptides encoded thereby, and ***antibodies***. Delta3 therapeutics, which are either antagonists or agonists of a Delta3 activity and which are capable of

modulating the growth and/or differentiation of a cell, e.g., endothelial cell, are also provided herein. Furthermore, methods for treating or preventing diseases associated with an aberrant Delta3 activity and/or associated with abnormal cellular growth and/or differentiation, e.g., neurological disease or vascular disease, such as Agenesis of the Corpus Callosum with Peripheral Neuropathy (ACCPN), as well as diagnostic methods for detecting these diseases are disclosed.

IN ***McCarthy, Sean A.***, San Diego, CA, UNITED STATES

AB The invention provides nucleic acids encoding Delta3 proteins. Also provided are derivatives of Delta3 nucleic acids, polypeptides encoded thereby, and ***antibodies***. Delta3 therapeutics, which are either antagonists or agonists of a Delta3 activity and which are capable of modulating the growth. . .

SUMM . . . et al., (1996) Nature 383: 707-710). CADASIL causes a type of stroke and dementia whose key features include recurrent subcortical ***ischemic*** events, progressive vascular dementia, craniofacial paralysis, migraine and mood disorders with severe depression (Chabriat et al. (1995) Lancet 346: 934-939).

SUMM . . . cause of dementia, after Alzheimer's disease. CADASIL causes a type of stroke and dementia whose key features include recurrent subcortical ***ischemic*** events, progressive vascular dementia, craniofacial paralysis, migraine and mood disorders with severe depression (Chabriat et al., (1995) Lancet 346: 934-939).. . .

SUMM . . . preparation, the immunogen being capable of eliciting an immune response specific for a Delta3 polypeptide, e.g., a humoral response, an ***antibody*** response and/or cellular response. In preferred embodiments, the immunogen comprises an antigenic determinant, e.g., a unique determinant, from the protein. . .

SUMM [0063] A still further aspect of the present invention features ***antibodies*** and ***antibody*** preparations specifically reactive with an epitope of the Delta3 protein. In preferred embodiments, the ***antibody*** specifically binds to an epitope of a polypeptide shown in SEQ ID NOs: 2, 25, 28, 30, 32, 34, 36,. . .

SUMM [0064] In yet a further aspect, the invention provides substantially purified ***antibodies*** or fragments thereof, including human or non-human ***antibodies*** or fragments thereof, which ***antibodies*** or fragments specifically bind to a polypeptide comprising an amino acid sequence selected from the group consisting of: the amino. . . of 6.times. SSC at 45.quadrature.C. and washing in 0.2.times. SSC, 0.1% SDS at 65.quadrature.C. In various embodiments, the substantially purified ***antibodies*** of the invention, or fragments thereof, can be human, non-human, chimeric and/or humanized ***antibodies***.

SUMM [0065] In another aspect, the invention provides human or non-human ***antibodies*** or fragments thereof, which ***antibodies*** or fragments specifically bind to a polypeptide comprising an amino acid sequence selected from the group consisting of: the amino. . . of 6.times. SSC at 45.degree. C. and washing in 0.2.times. SSC, 0.1% SDS at 65.degree. C. With respect to non-human ***antibodies***, such ***antibodies*** can be goat, mouse, sheep, horse, chicken, rabbit, or rat ***antibodies***. Alternatively, the non-human ***antibodies*** of the invention can be chimeric and/or humanized ***antibodies***. In addition, the human and non-human ***antibodies*** of the invention can be polyclonal ***antibodies*** or monoclonal ***antibodies***.

SUMM [0066] In still a further aspect, the invention provides monoclonal ***antibodies*** or fragments thereof, which ***antibodies*** or fragments specifically bind to a polypeptide comprising an amino acid sequence selected from the group consisting of: the amino. . . of hybridization of 6.times. SSC at 45.degree. C. and washing in 0.2.times. SSC, 0.1% SDS at 65.degree. C. The monoclonal ***antibodies*** can be human, humanized, chimeric and/or non-human ***antibodies***.

SUMM [0067] In a particularly preferred embodiment, the substantially purified ***antibodies*** or fragments thereof, the human and non-human ***antibodies*** or fragments thereof, and/or monoclonal ***antibodies*** or fragments thereof, of the invention specifically bind to an extracellular domain of the amino acid sequence of SEQ ID. . . the cDNA of a clone deposited with the ATCC.RTM. as Accession Number 98348. Preferably, the extracellular domain to which the ***antibody***, or fragment thereof, binds comprises amino acid residues 1-529 of SEQ ID NO: 2 of human Delta3, or amino acid. . .

SUMM [0068] Any of the ***antibodies*** of the invention can be conjugated to a therapeutic moiety or to a detectable substance. Non-limiting examples of detectable substances that can be conjugated to the ***antibodies*** of the invention are an enzyme, a prosthetic group, a fluorescent material, a luminescent material, a bioluminescent material, and a. . .

SUMM [0069] The invention also provides a kit containing an ***antibody*** of the invention and instructions for use. Such kits can also comprise an ***antibody*** of the invention conjugated to a detectable substance and instructions for use. Still another aspect of the invention is a pharmaceutical composition comprising an ***antibody*** of the invention and a pharmaceutically acceptable carrier. In preferred embodiments, the pharmaceutical composition contains an ***antibody*** of the invention, a therapeutic moiety, and a pharmaceutically acceptable carrier.

SUMM [0070] In addition, the polypeptides of the invention or biologically active portions thereof, or ***antibodies*** of the invention, can be incorporated into pharmaceutical compositions, which optionally include pharmaceutically acceptable carriers.

SUMM . . . of the invention such that activity or expression in the cell is modulated. In one embodiment, the agent is an ***antibody*** that specifically binds to a polypeptide of the invention.

SUMM . . . protein of the invention, or, alternatively, a nucleic acid of the invention. In other embodiments, the modulator is a peptide, ***antibody***, peptidomimetic, or other small organic molecule.

SUMM [0087] In alternate embodiments, the diagnostic methods comprise determining the level of a Delta3 protein in an immunoassay using an ***antibody*** which is specifically immunoreactive with a wildtype or mutant Delta3 protein.

SUMM . . . whether presently known or inherent. Antigenic functions include possession of an epitope or antigenic site that is capable of binding ***antibodies*** raised against a naturally-occurring or denatured Delta3 polypeptide or fragment thereof. Accordingly, an activity of a Delta3 protein can be. . .

SUMM [0098] The term " ***antibody*** " as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen. . . such as a polypeptide of the invention, e.g., an epitope of a polypeptide of the invention. A molecule The term " ***antibody*** "

as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen. . . Examples of immunologically active portions of immunoglobulin molecules include F(ab) and F(ab').sub.2 fragments which can be generated by treating the ***antibody*** with an enzyme such as pepsin. The invention provides polyclonal and monoclonal ***antibodies***. The term "monoclonal ***antibody***" or "monoclonal ***antibody*** composition", as used herein, refers to a population of ***antibody*** molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope.

SUMM [0103] The term "Delta3 therapeutic" refers to various compositions of Delta3 modulators (e.g., agonists or antagonists), such as polypeptides, ***antibodies***, peptidomimetics, small molecules and nucleic acids which are capable of mimicking or potentiating (agonizing) or inhibiting suppressing (antagonizing) Delta3 expression,. . .

DETD [0152] Accordingly, certain aspects of the present invention relate to Delta3 proteins, nucleic acid molecules encoding Delta3 proteins, ***antibodies*** immunoreactive with Delta3 proteins, and preparations of such compositions. In addition, drug discovery assays are provided for identifying agents that. . .

DETD . . . receptors or antigens expressed on a selected cell surface, e.g., by linking the antisense nucleic acid molecules to peptides or ***antibodies*** which bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using.

DETD . . . intact tissue and tissue samples for the presence (or absence) of Delta-encoding transcripts. Similar to the diagnostic uses of anti-Delta3 ***antibodies***, the use of probes directed to Delta3 messages, or to genomic Delta3 sequences, can be used for both predictive and. . .

DETD . . . into the tissue site, or modified antisense molecules, designed to target the desired cells (e.g., antisense linked to peptides or ***antibodies*** that specifically bind receptors or antigens expressed on the target cell surface) can be administered systematically.

DETD . . . techniques known in the art for purifying proteins including ion-exchange chromatography, gel filtration chromatography, ultrafiltration, electrophoresis, and immunoaffinity purification with ***antibodies*** specific for such peptide. In a preferred embodiment, the recombinant Delta3 polypeptide is a fusion protein containing a domain which. . .

DETD . . . form of a viral particle. The nucleic acid sequences corresponding to the portion of a subject Delta3 protein to which ***antibodies*** are to be raised can be incorporated into a fusion gene construct which includes coding sequences for a late vaccinia. .

DETD [0318] 4.3.3. ***Antibodies***

DETD [0319] Another aspect of the invention pertains to an ***antibody*** that binds to a Delta3 protein; that is, to ***antibodies*** directed against a polypeptide of the invention. For example, by using immunogens derived from a Delta3 protein, e.g., based on the cDNA sequences, anti-protein/anti-peptide antisera or monoclonal ***antibodies*** can be made by standard protocols (See, for example, ***Antibodies*** : A Laboratory Manual ed. by Harlow and Lane (Cold Spring Harbor Press: 1988)). A mammal, such as a mouse, a. . . an

immunogenic form of the peptide (e.g., a Delta3 polypeptide or an antigenic fragment which is capable of eliciting an ***antibody*** response, or a fusion protein as described above). Techniques for conferring immunogenicity on a protein or peptide include conjugation to. . . Delta3 protein can be administered in the presence of adjuvant. The progress of immunization can be monitored by detection of ***antibody*** titers in plasma or serum. Standard ELISA or other immunoassays can be used with the immunogen as antigen to assess the levels of ***antibodies***.

DETD . . . an animal with an antigenic preparation of a Delta3 polypeptide, anti-Delta3 antisera can be obtained and, if desired, polyclonal anti-Delta3 ***antibodies*** isolated from the serum. To produce monoclonal ***antibodies***, ***antibody***-producing cells (lymphocytes) can be harvested from an immunized animal and fused by standard somatic cell fusion procedures with immortalizing cells. . . B cell hybridoma technique (Kozbar et al., (1983) Immunology Today, 4: 72), and the EBV-hybridoma technique to produce human monoclonal ***antibodies*** (Cole et al., (1985) Monoclonal ***Antibodies*** and Cancer Therapy, Alan R. Liss, Inc. pp. 77-96). Hybridoma cells can be screened immunochemically for production of ***antibodies*** specifically reactive with a Delta3 polypeptide of the present invention and monoclonal ***antibodies*** isolated from a culture comprising such hybridoma cells. In one embodiment anti-human Delta3 ***antibodies*** specifically react with the proteins encoded by the DNA of ATCC.RTM. Deposit Accession Number 98348.

DETD [0321] ***Antibodies*** can be fragmented using conventional techniques and the fragments screened for utility in the same manner as described above for whole ***antibodies***. For example, F(ab)2 fragments can be generated by treating ***antibody*** with pepsin. The resulting F(ab)2 fragment can be treated to reduce disulfide bridges to produce Fab fragments. The ***antibody*** of the present invention is further intended to include bispecific and chimeric molecules having affinity for a Delta3 protein conferred by at least one CDR region of the ***antibody***.

DETD [0322] ***Antibodies*** which specifically bind Delta3 epitopes can also be used in immunohistochemical staining of tissue samples in order to evaluate the abundance and pattern of expression of each of the subject Delta3 polypeptides. Anti-Delta3 ***antibodies*** can be used diagnostically in immuno-precipitation and immuno-blotting to detect and evaluate Delta3 protein levels in tissue as part of. . . spinal fluid or amniotic fluid, or can be measured in tissue, such as produced by biopsy. Diagnostic assays using anti-Delta3 ***antibodies*** can include, for example, immunoassays designed to aid in early diagnosis of a neurodegenerative disorder, particularly ones which are manifest at birth. Diagnostic assays using anti-Delta3 polypeptide ***antibodies*** can also include immunoassays designed to aid in early diagnosis and phenotyping neurodegenerative, neoplastic or hyperplastic disorders.

DETD [0323] Another application of anti-Delta3 ***antibodies*** of the present invention is in the immunological screening of cDNA libraries constructed in expression vectors such as .lambda.gt11, .lambda.gt18-23,. . . e.g., other orthologs of a particular Delta3 protein or other paralogs from the same species, can then be detected with ***antibodies***, as, for example, reacting nitrocellulose filters lifted from infected plates with anti-Delta3 ***antibodies***.

. Positive phage detected by this assay can then be isolated from the infected plate. Thus, the presence of Delta3 homologs. . .

DETD [0324] Polyclonal ***antibodies*** can be prepared as described above by immunizing a suitable subject with a polypeptide of the invention as an immunogen. Preferred polyclonal ***antibody*** compositions are ones that have been selected for ***antibodies*** directed against a polypeptide or polypeptides of the invention. Particularly preferred polyclonal ***antibody*** preparations are ones that contain only ***antibodies*** directed against a polypeptide or polypeptides of the invention. Particularly preferred immunogen compositions are those that contain no other human. . . of a polypeptide of the invention. In such a manner, the only human epitope or epitopes recognized by the resulting ***antibody*** compositions raised against this immunogen will be present as part of a polypeptide or polypeptides of the invention.

DETD [0325] The ***antibody*** titer in the immunized subject can be monitored over time by standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using immobilized polypeptide. If desired, the ***antibody*** molecules can be isolated from the mammal (e.g., from the blood) and further purified by well-known techniques, such as protein A chromatography to obtain the IgG fraction. Alternatively, ***antibodies*** specific for a protein or polypeptide of the invention can be selected for (e.g., partially purified) or purified by, e.g., . . . to a solid support such as, for example, a chromatography column. The column can then be used to affinity purify ***antibodies*** specific for the proteins of the invention from a sample containing ***antibodies*** directed against a large number of different epitopes, thereby generating a substantially purified ***antibody*** composition, i.e., one that is substantially free of contaminating ***antibodies***. By a substantially purified ***antibody*** composition is meant, in this context, that the ***antibody*** sample contains at most only 30% (by dry weight) of contaminating ***antibodies*** directed against epitopes other than those on the desired protein or polypeptide of the invention, and preferably at most 20%,. . . yet more preferably at most 10%, and most preferably at most 5% (by dry weight) of the sample is contaminating ***antibodies***. A purified ***antibody*** composition means that at least 99% of the ***antibodies*** in the composition are directed against the desired protein or polypeptide of the invention.

DETD [0326] At an appropriate time after immunization, e.g., when the specific ***antibody*** titers are highest, ***antibody*** -producing cells can be obtained from the subject and used to prepare monoclonal ***antibodies*** by standard techniques, such as the hybridoma technique originally described by Kohler and Milstein (1975) Nature 256:495-497, the human B cell hybridoma technique (Kozbor et al. (1983) Immunol. Today 4:72), the EBV-hybridoma technique (Cole et al. (1985), Monoclonal ***Antibodies*** and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96) or trioma techniques. The technology for producing hybridomas is well known. . . in Immunology (1994) Coligan et al. (eds.) John Wiley & Sons, Inc., New York, N.Y.). Hybridoma cells producing a monoclonal ***antibody*** of the invention are detected by screening the hybridoma culture supernatants for ***antibodies*** that bind the polypeptide of interest, e.g., using a standard ELISA assay.

DETD [0327] Alternative to preparing monoclonal ***antibody*** -secreting hybridomas, a monoclonal ***antibody*** directed against a polypeptide of the invention can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (e.g., an ***antibody*** phage display library) with the polypeptide of interest. Kits for generating and screening phage display libraries are commercially available (e.g., the Pharmacia Recombinant Phage ***Antibody*** System, Catalog NO: 27-9400-01; and the Stratagene SurfZAP Phage Display Kit, Catalog NO: 240612). Additionally, examples of methods and reagents particularly amenable for use in generating and screening ***antibody*** display library can be found in, for example, U.S. Pat. No. 5,223,409; PCT Publication No: WO 92/18619; PCT Publication No: . . . Publication No: WO 92/09690; PCT Publication No: WO 90/02809; Fuchs et al. (1991) Bio/Technology 9:1370-1372; Hay et al. (1992) Hum. ***Antibod***. Hybridomas 3:81-85; Huse et al. (1989) Science 246:1275-1281; Griffiths et al. (1993) EMBO J. 12:725-734.

DETD [0328] Additionally, recombinant ***antibodies***, such as chimeric and humanized monoclonal ***antibodies***, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the invention. A chimeric ***antibody*** is a molecule in which different portions are derived from different animal species, such as those having a variable region. . . No. 4,816,567; and Boss et al., U.S. Pat. No. 4,816,397, which are incorporated herein by reference in their entirety.) Humanized ***antibodies*** are ***antibody*** molecules from non-human species having one or more complementarily determining regions (CDRs) from the non-human species and a framework region from a human immunoglobulin molecule. (See, e.g., Queen, U.S. Pat. No. 5,585,089.) Such chimeric and humanized monoclonal ***antibodies*** can be produced by recombinant DNA techniques known in the art, for example using methods described in PCT Publication NO: . . .

DETD [0329] Completely human ***antibodies*** are particularly desirable for therapeutic treatment of human patients. Such ***antibodies*** can be produced, for example, using transgenic mice which are incapable of expressing endogenous immunoglobulin heavy and light chains genes, . . . in the normal fashion with a selected antigen, e.g., all or a portion of a polypeptide of the invention. Monoclonal ***antibodies*** directed against the antigen can be obtained using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice. . . switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA and IgE ***antibodies***. For an overview of this technology for producing human ***antibodies***, see Lonberg and Huszar (1995, Int. Rev. Immunol. 13:65-93). For a detailed discussion of this technology for producing human ***antibodies*** and human monoclonal ***antibodies*** and protocols for producing such ***antibodies***, see, e.g., U.S. Pat. No. 5,625,126; U.S. Pat. No. 5,633,425; U.S. Pat. No. 5,569,825; U.S. Pat. No. 5,661,016; and U.S. Pat. No. 5,545,806. In addition, companies such as Abgenix, Inc. (Freemont, Calif.), can be engaged to provide human ***antibodies*** directed against a selected antigen using technology similar to that described above.

DETD [0330] Completely human ***antibodies*** which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal ***antibody***, e.g., a murine ***antibody***, is used to guide

the selection of a completely human ***antibody*** recognizing the same epitope. (Jespers et al. (1994) Bio/technology 12:899-903).

DETD [0331] An ***antibody*** directed against a polypeptide of the invention (e.g., monoclonal ***antibody***) can be used to isolate the polypeptide by standard techniques, such as affinity chromatography or immunoprecipitation. Moreover, such an ***antibody*** can be used to detect the protein (e.g., in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the polypeptide. The ***antibodies*** can also be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, e.g., to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the ***antibody*** to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and. . .

DETD [0332] Further, an ***antibody*** (or fragment thereof) can be conjugated to a therapeutic moiety such as a cytotoxin, a therapeutic agent or a radioactive. . .

DETD [0334] Techniques for conjugating such therapeutic moiety to ***antibodies*** are well known, see, e.g., Arnon et al., "Monoclonal ***Antibodies*** For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal ***Antibodies*** And Cancer Therapy, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom et al., " ***Antibodies*** For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, " ***Antibody*** Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal ***Antibodies*** '84: Biological And Clinical Applications, Pinchera et al. (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled ***Antibody*** In Cancer Therapy", in Monoclonal ***Antibodies*** For Cancer Detection And Therapy, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985), and Thorpe et al., "The Preparation And Cytotoxic Properties Of ***Antibody*** -Toxin Conjugates", Immunol. Rev., 62:119-58 (1982).

DETD [0335] Alternatively, an ***antibody*** can be conjugated to a second ***antibody*** to form an ***antibody*** heteroconjugate as described by Segal in U.S. Pat. No. 4,676,980, incorporated herein by reference in its entirety.

DETD [0336] Accordingly, in one aspect, the invention provides substantially purified ***antibodies*** or fragment thereof, and human and non-human ***antibodies*** or fragments thereof, which ***antibodies*** or fragments specifically bind to a polypeptide comprising an amino acid sequence selected from the group consisting of: the amino. . . of 6.times. SSC at 45.quadrature.C. and washing in 0.2.times. SSC, 0.1% SDS at 65.quadrature.C. In various embodiments, the substantially purified ***antibodies*** of the invention, or fragments thereof, can be human, non-human, chimeric and/or humanized ***antibodies*** .

DETD [0337] In another aspect, the invention provides human and non-human ***antibodies*** or fragments thereof, which ***antibodies*** or fragments specifically bind to a polypeptide comprising an amino acid sequence selected from the group consisting of: the amino. . . under conditions of hybridization of 6.times. SSC at 45.quadrature.C. and washing in 0.2.times. SSC, 0.1% SDS at 65.quadrature.C. Such non-human ***antibodies*** can be goat, mouse, sheep, horse, chicken, rabbit, or

rat ***antibodies***. Alternatively, the non-human ***antibodies*** of the invention can be chimeric and/or humanized ***antibodies***. In addition, the non-human ***antibodies*** of the invention can be polyclonal ***antibodies*** or monoclonal ***antibodies***.

DETD [0338] In still a further aspect, the invention provides monoclonal ***antibodies*** or fragments thereof, which ***antibodies*** or fragments specifically bind to a polypeptide comprising an amino acid sequence selected from the group consisting of: the amino. . . under conditions of hybridization of 6.times. SSC at 45.quadrature.C. and washing in 0.2.times. SSC, 0.1% SDS at 65.quadrature.C. The monoclonal ***antibodies*** can be human, humanized, chimeric and/or non-human ***antibodies***.

DETD [0339] The substantially purified ***antibodies*** or fragments thereof specifically bind to a signal peptide, a secreted sequence, an extracellular domain, a transmembrane or a cytoplasmic domain cytoplasmic membrane of a polypeptide of the invention. In a particularly preferred embodiment, the substantially purified ***antibodies*** or fragments thereof, the human and non-human ***antibodies*** or fragments thereof, and/or the monoclonal ***antibodies*** or fragments thereof, of the invention specifically bind to a secreted sequence or an extracellular domain of the amino acid. . . 30, 32, 34, 36, 38, 40, 42, 44 or 46. Preferably, the secreted sequence or extracellular domain to which the ***antibody***, or fragment thereof, binds comprises from about amino acids 1-529 or 18-529 of SEQ ID NO: 2, or from amino. . .

DETD [0340] Any of the ***antibodies*** of the invention can be conjugated to a therapeutic moiety or to a detectable substance. Non-limiting examples of detectable substances that can be conjugated to the ***antibodies*** of the invention are an enzyme, a prosthetic group, a fluorescent material, a luminescent material, a bioluminescent material, and a. . .

DETD [0341] The invention also provides a kit containing an ***antibody*** of the invention, and instructions for use. In another embodiment, a kit comprising an ***antibody*** of the invention conjugated to a detectable substance and instructions for use. Still another aspect of the invention is a pharmaceutical composition comprising an ***antibody*** of the invention and a pharmaceutically acceptable carrier. In preferred embodiments, the pharmaceutical composition contains an ***antibody*** of the invention, a therapeutic moiety, and a pharmaceutically acceptable carrier.

DETD [0342] Still another aspect of the invention is a method of making an ***antibody*** that binds, that is, is directed against, Delta3, the method comprising immunizing a mammal with a polypeptide. The polypeptide used. . . washing in 0.2.times. SSC, 0.1% SDS at 65.quadrature.C. After immunization, a sample is collected from the mammal that contains an ***antibody*** that specifically recognizes Delta3. Preferably, the polypeptide is recombinantly produced using a non-human host cell. Optionally, the ***antibodies*** can be further purified from the sample using techniques well known to those of skill in the art. The method can further comprise producing a monoclonal ***antibody***-producing cell from the cells of the mammal. Optionally, ***antibodies*** are collected from the ***antibody***-producing cell.

DETD . . . of childhood (Fazio-Londe syndrome), poliomyelitis, and

hereditary motorsensory neuropathy (Charcot-Marie-Tooth disease), spinal cord injuries, brain injuries, lesions associated with surgery, ***ischemic*** lesions, malignant lesions, infectious lesions.

DETD . . . coronary artery disease, coronary revascularization, coronary restenosis, ventricular thromboembolism, atherosclerosis, coronary artery disease (e.g., arterial occlusive disorders), plaque formation, cardiac ***ischemia***, including complications related to coronary procedures, such as percutaneous coronary artery angioplasty (balloon angioplasty) procedures) can be ameliorated. With respect. . .

DETD . . . tumors. Alternatively, cells expressing or over-expressing Delta3 can be targeted for lysis, by, for example, targeting to the cell an ***antibody*** binding specifically to a Delta3 protein linked to a cytotoxic peptide.

DETD [0375] The nucleic acid molecules, polypeptides, and ***antibodies*** (also referred to herein as "active compounds") of the invention can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the nucleic acid molecule, protein, or ***antibody*** and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all. . .

DETD [0379] Sterile injectable solutions can be prepared by incorporating the active compound (e.g., a polypeptide or ***antibody***) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed. . .

DETD . . . be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal ***antibodies*** to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to. . .

DETD [0387] For ***antibodies***, the preferred dosage is 0.1 mg/kg to 100 mg/kg of body weight (generally 10 mg/kg to 20 mg/kg). If the ***antibody*** is to act in the brain, a dosage of 50 mg/kg to 100 mg/kg is usually appropriate. Generally, partially human ***antibodies*** and fully human ***antibodies*** have a longer half-life within the human body than other ***antibodies***. Accordingly, lower dosages and less frequent administration is often possible. Modifications such as lipidation can be used to stabilize ***antibodies*** and to enhance uptake and tissue penetration (e.g., into the brain). A method for lipidation of ***antibodies*** is described by Cruikshank et al. ((1997) J. Acquired Immune Deficiency Syndromes and Human Retrovirology 14:193).

DETD . . . described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or ***antibody*** reagent described herein, which may be conveniently used, e.g., in clinical settings to diagnose patients exhibiting symptoms or family history. . .

DETD [0416] ***Antibodies*** directed against wild type or mutant Delta3 proteins, which are discussed, above, may also be used in disease diagnostics and. . . herein may also be such as those described in Harlow and Lane, for example, (Harlow, E. and Lane, D., 1988, " ***Antibodies*** : A Laboratory Manual", Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.).

DETD [0417] This can be accomplished, for example, by immunofluorescence techniques employing a fluorescently labeled ***antibody*** (see below) coupled with light microscopic, flow cytometric, or fluorimetric

detection. The ***antibodies*** (or fragments thereof) useful in the present invention may, additionally, be employed histologically, as in immunofluorescence or immunoelectron microscopy, for. . . proteins. In situ detection may be accomplished by removing a histological specimen from a patient, and applying thereto a labeled ***antibody*** of the present invention. The ***antibody*** (or fragment) is preferably applied by overlaying the labeled ***antibody*** (or fragment) onto a biological sample. Through the use of such a procedure, it is possible to determine not only. . .

DETD [0418] Often a solid phase support or carrier is used as a support capable of binding an antigen or an ***antibody***. Well-known supports or carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, gabbros, and magnetite.. . . have virtually any possible structural configuration so long as the coupled molecule is capable of binding to an antigen or ***antibody***. Thus, the support configuration may be spherical, as in a bead, or cylindrical, as in the inside surface of a. . . strip, etc. Preferred supports include polystyrene beads. Those skilled in the art will know many other suitable carriers for binding ***antibody*** or antigen, or will be able to ascertain the same by use of routine experimentation.

DETD [0419] One means for labeling an anti-Delta3 protein specific ***antibody*** is via linkage to an enzyme and use in an enzyme immunoassay (EIA) (Voller, "The Enzyme Linked Immunosorbent Assay (ELISA)",. . . Raton, Fla., 1980; Ishikawa, et al., (eds.) Enzyme Immunoassay, Kaku Shoin, Tokyo, 1981). The enzyme which is bound to the ***antibody*** will react with an appropriate substrate, preferably a chromogenic substrate, in such a manner as to produce a chemical moiety. . . be detected, for example, by spectrophotometric, fluorimetric or by visual means. Enzymes which can be used to detectably label the ***antibody*** include, but are not limited to, malate dehydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, yeast alcohol dehydrogenase, alpha-glycerophosphate, dehydrogenase, triose phosphate isomerase,. . .

DETD [0420] Detection may also be accomplished using any of a variety of other immunoassays. For example, by radioactively labeling the ***antibodies*** or ***antibody*** fragments, it is possible to detect fingerprint gene wild type or mutant peptides through the use of a radioimmunoassay (RIA). . .

DETD [0421] It is also possible to label the ***antibody*** with a fluorescent compound. When the fluorescently labeled ***antibody*** is exposed to light of the proper wave length, its presence can then be detected due to fluorescence. Among the. . .

DETD [0422] The ***antibody*** can also be detectably labeled using fluorescence emitting metals such as ¹⁵²Eu, or others of the lanthanide series. These metals can be attached to the ***antibody*** using such metal chelating groups as diethylenetriaminepentaacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA).

DETD [0423] The ***antibody*** also can be detectably labeled by coupling it to a chemiluminescent compound. The presence of the chemiluminescent-tagged ***antibody*** is then determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of. . .

DETD [0424] Likewise, a bioluminescent compound may be used to label the ***antibody*** of the present invention. Bioluminescence is a type of

chemiluminescence found in biological systems in, which a catalytic protein increases. . .

DETD . . . (e.g., biotinylation kit, Pierce Chemicals, Rockford, Ill.), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, ***antibodies*** reactive with Delta3 but which do not interfere with binding of upstream or downstream elements can be derivatized to the wells of the plate, and Delta3 trapped in the wells by ***antibody*** conjugation. As above, preparations of a Delta-binding protein and a test compound are incubated in the Delta-presenting wells of the. . . methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using ***antibodies*** reactive with the Delta3 binding element, or which are reactive with Delta3 protein and compete with the binding element; as. . .

DETD [0440] For processes which rely on immunodetection for quantitating one of the proteins trapped in the complex, ***antibodies*** against the protein, such as anti-Delta3 ***antibodies***, can be used. Alternatively, the protein to be detected in the complex can be "epitope tagged" in the form of a fusion protein which includes, in addition to the Delta3 sequence, a second polypeptide for which ***antibodies*** are readily available (e.g., from commercial sources). For instance, the GST fusion proteins described above can also be used for quantification of binding using ***antibodies*** against the GST moiety. Other useful epitope tags include myc-epitopes (e.g., see Ellison et al. (1991) J Biol Chem 266:21150-21157). . .

DETD [0461] A polypeptide and fragments and sequences thereof and ***antibodies*** specific thereto can be used to map the location of the gene encoding the polypeptide on a chromosome. This mapping. . .

DETD . . . described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or ***antibody*** reagent described herein, which may be conveniently used, e.g., in clinical settings to diagnose patients exhibiting symptoms or family history. . .

DETD . . . analysis, using a probe that is complementary to at least a portion of the transgene. Western blot analysis using an ***antibody*** against the protein encoded by the transgene may be employed as an alternative or additional method for screening for the. . .

DETD . . . expression of the Delta3 gene knocked out in various tissues of the offspring by probing the Western blot with an ***antibody*** against the particular Delta3 protein, or an ***antibody*** against the marker gene product, where this gene is expressed. Finally, in situ analysis (such as fixing the cells and labeling with ***antibody***) and/or FACS (fluorescence activated cell sorting) analysis of various cells from the offspring can be conducted using suitable ***antibodies*** to look for the presence or absence of the knockout construct gene product.

DETD . . . resolved on an SDS-polyacrylamide gel. The proteins were then transferred onto a nitrocellulose membrane and probed with an anti-Troponin T ***antibody*** (Sigma, 1:200). A secondary incubation with an anti-mouse ***antibody*** conjugated to horseradish peroxidase allowed for detection using chemiluminescence reagents (Amersham). When cells NIH3T3 cells containing the empty vector were. . .

DETD . . . cells were washed three times in staining buffer and bound protein was detected by incubating the cells with FITC-conjugated secondary ***antibody*** (anti-human hIgG1) for 30 min on ice. Cells were analyzed by flow cytometry on a FACSCalibur.

DETD . . . surface and the other protein is labeled, e.g., such as by tagging the protein with an epitope, for which an ***antibody*** is available (e.g., FLAG epitope, available from International Biotechnologies, Inc.). As a non-limiting example of an assay, the Delta3 protein. . .

DETD . . . wells is determined. The amount of bound protein can be determined by incubating the wells with an anti-tag, e.g., anti-myc, ***antibody***, which can then be detected by enzyme immunoassay. The amount of bound protein is then determined by determining the optical.

DETD . . . polypeptide is not labeled and is detected upon binding the cell by a second Delta3 binding protein, such as an ***antibody***. A lower amount of Delta3 binding protein in a well that contained a test compound relative to a well that. . .

CLM What is claimed is:

21. An ***antibody*** which selectively binds to a polypeptide of claim 13.
22. The ***antibody*** of claim 21, which is a monoclonal ***antibody***.
23. The ***antibody*** of claim 22, comprising an immunologically active portion selected from the group consisting of: a) an scFV fragment; b) a. . .
24. The ***antibody*** of claim 22, wherein the ***antibody*** is selected from the group consisting of: a) a chimeric ***antibody***; b) a humanized ***antibody***; c) a human ***antibody***; d) a non-human ***antibody***; and e) a single chain ***antibody***.
27. The method of claim 26, wherein the compound which binds to the polypeptide is an ***antibody***.

L9 ANSWER 3 OF 9 USPATFULL on STN

AN 2003:207274 USPATFULL

TI Novel genes encoding proteins having prognostic, diagnostic, preventive, therapeutic and other uses

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PI US 2003143632 A1 20030731

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RLI Continuation of Ser. No. US 2001-802582, filed on 8 Mar 2001, ABANDONED

Continuation-in-part of Ser. No. US 1998-128709, filed on 4 Aug 1998,

ABANDONED Continuation-in-part of Ser. No. US 1998-130491, filed on 6

Aug 1998, GRANTED, Pat. No. US 6416974 Continuation-in-part of Ser. No.

US 1998-130491, filed on 6 Aug 1998, GRANTED, Pat. No. US 6416974

PRAI US 1997-54645P 19970804 (60)

US 1997-54966P 19970806 (60)

US 1997-58108P 19970905 (60)
US 1997-54966P 19970806 (60)
US 1997-58108P 19970905 (60)
US 1997-54966P 19970806 (60)
US 1997-58108P 19970905 (60)

DT Utility

FS APPLICATION

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CLMN Number of Claims: 39

ECL Exemplary Claim: 1

DRWN 38 Drawing Page(s)

LN.CNT 4601

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention relates to Tango-73, Tango-74, Tango-76, Tango-78, and Tango-83 polypeptides, nucleic acid molecules encoding Tango-73, Tango-74, Tango-76, Tango-78, and Tango-83, and uses thereof. The invention provides isolated nucleic acids encoding a variety of proteins having diagnostic, preventive, therapeutic, and other uses. These nucleic and proteins are useful for diagnosis, prevention, and therapy of a number of human and other animal disorders. The invention also provides antisense nucleic acid molecules, expression vectors containing the nucleic acid molecules of the invention, host cells into which the expression vectors have been introduced, and non-human transgenic animals in which a nucleic acid molecule of the invention has been introduced or disrupted. The invention still further provides isolated polypeptides, fusion polypeptides, antigenic peptides and ***antibodies***. Diagnostic, screening, and therapeutic methods using compositions of the invention are also provided. The nucleic acids and polypeptides of the present invention are useful as modulating agents in regulating a variety of cellular processes.

IN ***McCarthy, Sean A.***, San Diego, CA, UNITED STATES

AB . . . of the invention has been introduced or disrupted. The invention still further provides isolated polypeptides, fusion polypeptides, antigenic peptides and ***antibodies***. Diagnostic, screening, and therapeutic methods using compositions of the invention are also provided. The nucleic acids and polypeptides of the . . .

SUMM [0012] The invention also features ***antibodies***, e.g., monoclonal, polyclonal, and engineered ***antibodies***, which specifically bind polypeptides of the invention. By "specifically binds" is meant an ***antibody*** that recognizes and binds to a particular antigen, e.g., a Tango-73, Tango-74, Tango-76, Tango-78, or Tango-83 polypeptide of the invention, . . .

SUMM . . . be used directly as diagnostic or therapeutic agents, or (in the case of a polypeptide) can be used to generate ***antibodies*** that, in turn, are therapeutically useful. Accordingly, expression vectors containing the nucleic acid molecules of the invention, cells transfected with these vectors, the polypeptides expressed, and ***antibodies*** generated (against either the entire polypeptide or an antigenic fragment thereof) are among the preferred embodiments.

SUMM . . . functional polypeptide is also considered within the scope of the invention if it serves as an antigen for production of ***antibodies*** that specifically bind to a polypeptide of the invention. In many cases, functional polypeptides retain one or more domains present. . .

SUMM . . . (i.e., molecules with a molecular weight below about 500);

large molecules (i.e., molecules with a molecular weight above about 500); ***antibodies*** that bind and "neutralize" polypeptides of the invention (as described below); polypeptides that compete with a native form of a . . . acid molecules and ribozymes). Agonists of nucleic acids or polypeptides of the invention also include small and large molecules, and ***antibodies*** other than neutralizing ***antibodies***.

SUMM . . . invention (i.e., Tango-73, Tango-74, Tango-76, Tango-78, or Tango-83). Such methods include: obtaining a biological sample; contacting the sample with an ***antibody*** that specifically binds to the protein under conditions that permit specific binding; and detecting any ***antibody***-protein complexes formed.

DETD . . . a protein is to be produced, for the generation of pharmaceutical compositions containing polypeptides of the invention or for raising ***antibodies*** to those polypeptides, vectors that are capable of directing the expression of high levels of fusion protein products that are. . .

DETD . . . the invention. These polypeptides can be prepared for a variety of uses, including but not limited to the generation of ***antibodies***, as reagents in diagnostic assays, for the identification of other cellular gene products or compounds that can modulate the activity. . .

DETD [0121] A fusion protein may be readily purified by utilizing an ***antibody*** specific for the fusion protein being expressed. For example, a system described by Janknecht et al. allows for the ready. .

DETD . . . tissue samples obtained from the animal, in situ hybridization analysis, and RT-PCR. Biological samples can also be evaluated immunocytochemically using ***antibodies*** specific for the product of the transgene of the invention. Samples of tissue expressing the gene of the invention can also be evaluated immunocytochemically using ***antibodies*** specific for the product of the transgene of the invention.

DETD Anti-Tango-73, Tango-74, Tango-76, Tango-78, or Tango-83
Antibodies

DETD [0131] Human polypeptides of the invention (or immunogenic fragments or analogs) can be used to raise ***antibodies*** useful in the invention; such polypeptides can be produced by recombinant techniques or synthesized (see, for example, "Solid Phase Peptide. . . such as KLH, as described in Ausubel et al., supra, mixed with an adjuvant, and injected into a host mammal. ***Antibodies*** can be purified by peptide antigen affinity chromatography.

DETD . . . peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol. Potentially useful human adjuvants include BCG (bacille Calmette-Guerin) and Corynebacterium parvum. Polyclonal ***antibodies*** are heterogeneous populations of ***antibody*** molecules that are contained in the sera of the immunized animals.

DETD [0133] ***Antibodies*** within the invention therefore include polyclonal ***antibodies*** and, in addition, monoclonal ***antibodies***, humanized or chimeric ***antibodies***, single chain ***antibodies***, Fab fragments, F(ab').sub.2 fragments, and molecules produced using a Fab expression library.

DETD [0134] Monoclonal ***antibodies***, which are homogeneous populations of ***antibodies*** to a particular antigen, can be prepared using the polypeptides of the invention described above and

standard hybridoma technology (see, . . . Kohler et al., Eur. J. Immunol. 6:511, 1976; Kohler et al., Eur. J. Immunol. 6:292, 1976; Hammerling et al., "Monoclonal ***Antibodies*** and T Cell Hybridomas," Elsevier, N.Y., 1981; Ausubel et al., supra).

DETD [0135] In particular, monoclonal ***antibodies*** can be obtained by any technique that provides for the production of ***antibody*** molecules by continuous cell lines in culture such as described in Kohler et al., Nature 256:495, 1975, and U.S. Pat. . . . 4:72, 1983; Cole et al., Proc. Natl. Acad. Sci. USA 80:2026, 1983), and the EBV-hybridoma technique (Cole et al., "Monoclonal ***Antibodies*** and Cancer Therapy," Alan R. Liss, Inc., pp. 77-96, 1983). Such ***antibodies*** can be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD and any subclass thereof. The hybridoma producing the . . .

DETD [0136] Once produced, polyclonal or monoclonal ***antibodies*** are tested for specific recognition of polypeptides of the invention by Western blot or immunoprecipitation analysis by standard methods, e.g., as described in Ausubel et al., supra. ***Antibodies*** that specifically recognize and bind to polypeptides of the invention are useful in the invention. For example, such ***antibodies*** can be used in an immunoassay to monitor the level of a polypeptide of the invention produced by a mammal. . .

DETD [0137] Preferably, ***antibodies*** of the invention are produced using fragments of the protein of the invention that lie outside highly conserved regions and. . .

DETD [0140] The ***antibodies*** can be used, for example, in the detection of the polypeptide of the invention in a biological sample as part of a diagnostic assay. ***Antibodies*** also can be used in a screening assay to measure the effect of a candidate compound on expression or localization of a polypeptide of the invention. Additionally, such ***antibodies*** can be used in conjunction with the gene therapy techniques described to, for example, evaluate normal and/or genetically engineered cells that express nucleic acids or polypeptides of the invention prior to their introduction into the patient. Such ***antibodies*** additionally can be used in a method for inhibiting abnormal activity of nucleic acids or polypeptides of the invention.

DETD [0141] In addition, techniques developed for the production of "chimeric ***antibodies*** " (Morrison et al., Proc. Natl. Acad. Sci. USA, 81:6851, 1984; Neuberger et al., Nature, 312:604, 1984; Takeda et al., Nature, 314:452, 1984) by splicing the genes from a mouse ***antibody*** molecule of appropriate antigen specificity together with genes from a human ***antibody*** molecule of appropriate biological activity can be used. A chimeric ***antibody*** is a molecule in which different portions are derived from different animal species, such as those having a variable region. . .

DETD [0142] Generally, partially human ***antibodies*** and fully human ***antibodies*** have a longer half-life within the human body than other ***antibodies***. Accordingly, lower dosages and less frequent administration are often possible. Modifications such as lipidation can be used to stabilize ***antibodies*** and to enhance uptake and tissue penetration (e.g., into the brain). A method for lipidation of ***antibodies*** is described by Cruikshank et al. ((1997) J. Acquired Immune Deficiency Syndromes and Human Retrovirology 14:193).

DETD [0143] Alternatively, techniques described for the production of single

chain ***antibodies*** (U.S. Pat. Nos. 4,946,778, 4,946,778, and 4,704,692) can be adapted to produce single chain ***antibodies*** against polypeptides of the invention. Single chain ***antibodies*** are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting. . .

DETD [0144] ***Antibody*** fragments that recognize and bind to specific epitopes can be generated by known techniques. For example, such fragments include but are not limited to F(ab').sub.2 fragments that can be produced by pepsin digestion of the ***antibody*** molecule, and Fab fragments that can be generated by reducing the disulfide bridges of F(ab').sub.2 fragments. Alternatively, Fab expression libraries. . .

DETD [0145] ***Antibodies*** to polypeptides of the invention can, in turn, be used to generate anti-idiotypic ***antibodies*** that resemble a portion of the protein of the invention using techniques well known to those skilled in the art (see, e.g., Greenspan et al., FASEB J. 7:437, 1993; Nissinoff, J. Immunol. 147:2429, 1991). For example, ***antibodies*** that bind to the protein of the invention and competitively inhibit the binding of a binding partner of the protein. . . partner binding domain of the protein and, therefore, bind and neutralize a binding partner of the protein. Such neutralizing anti-idiotypic ***antibodies*** or Fab fragments of such anti-idiotypic ***antibodies*** can be used in therapeutic regimens.

DETD [0146] ***Antibodies*** can be humanized by methods known in the art. For example, monoclonal ***antibodies*** with a desired binding specificity can be commercially humanized (Scotgene, Scotland; Oxford Molecular, Palo Alto, Calif.). Fully human ***antibodies***, such as those expressed in transgenic animals are also features of the invention (Green et al., Nature Genetics 7:13-21, 1994;. . .

DETD [0147] The methods described herein in which anti-polypeptide-of-the-invention ***antibodies*** are employed may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one specific polypeptide-of-the-invention ***antibody*** reagent described herein, which may be conveniently used, for example, in clinical settings, to diagnose patients exhibiting symptoms of disorders. . .

DETD [0148] An ***antibody*** (or fragment thereof) can be conjugated to a therapeutic moiety such as a cytotoxin, a therapeutic agent, or a radioactive. . .

DETD [0149] Conjugated ***antibodies*** of the invention can be used for modifying a given biological response, the drug moiety not being limited to classical. . .

DETD [0150] Techniques for conjugating a therapeutic moiety to an ***antibody*** are well known (see, e.g., Arnon et al., 1985, "Monoclonal ***Antibodies*** For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal ***Antibodies*** And Cancer Therapy, Reisfeld et al., Eds., Alan R. Liss, Inc. pp. 243-256; Hellstrom et al., 1987, " ***Antibodies*** For Drug Delivery", in Controlled Drug Delivery, 2nd ed., Robinson et al., Eds., Marcel Dekker, Inc., pp. 623-653; Thorpe, 1985, " ***Antibody*** Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal ***Antibodies*** '84: Biological And Clinical Applications, Pinchera et al., Eds., pp. 475-506; "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled ***Antibody*** In Cancer Therapy", in Monoclonal ***Antibodies*** For Cancer Detection And Therapy, Baldwin et al., Eds., Academic Press, pp. 303-316, 1985; and Thorpe et al., 1982,

Immunol. Rev., 62:119-158). Alternatively, an ***antibody*** can be conjugated to a second ***antibody*** to form an ***antibody*** heteroconjugate as described by Segal in U.S. Pat. No. 4,676,980.

DETD . . . into the tissue site, or modified antisense molecules, designed to target the desired cells (e.g., antisense linked to peptides or ***antibodies*** that specifically bind receptors or antigens expressed on the target cell surface) can be administered systemically.

DETD . . . kilogram to about 500 milligrams per kilogram, or about 1 milligram per kilogram to about 50 milligrams per kilogram). For ***antibodies***, examples of dosages are from about 0.1 milligram per kilogram to 100 milligrams per kilogram of body weight (generally 10 milligrams per kilogram to 20 milligrams per kilogram). If the ***antibody*** is to act in the brain, a dosage of 50 milligrams per kilogram to 100 milligrams per kilogram is usually. . .

DETD . . . the invention. These methods include, for example, screening expression libraries, in a manner similar to the well known technique of ***antibody*** probing of .lambda.gt11 libraries, using labeled polypeptide of the invention or a fusion protein of the invention, e.g., a polypeptide. . .

DETD [0186] The nucleic acid molecules, proteins, protein homologs, and ***antibodies*** described herein can be used in one or more of the following methods: a) screening assays; b) detection assays (e.g., . . . a protein of the invention which has decreased or aberrant activity compared to the wild type protein. In addition, the ***antibodies*** of the invention can be used to detect and isolate a protein of the and modulate activity of a protein. . .

DETD . . . (e.g., biotinylation kit, Pierce Chemicals; Rockford, Ill.), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, ***antibodies*** reactive with the polypeptide of the invention or target molecules but which do not interfere with binding of the polypeptide. . . derivatized to the wells of the plate, and unbound target or polypeptide of the invention trapped in the wells by ***antibody*** conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using ***antibodies*** reactive with the polypeptide of the invention or target molecule, as well as enzyme-linked assays which rely on detecting an. . .

DETD [0213] A polypeptide and fragments and sequences thereof and ***antibodies*** specific thereto can be used to map the location of the gene encoding the polypeptide on a chromosome. This mapping. . .

DETD [0228] A preferred agent for detecting a polypeptide of the invention is an ***antibody*** capable of binding to a polypeptide of the invention, preferably an ***antibody*** with a detectable label.

Antibodies can be polyclonal, or more preferably, monoclonal. An intact ***antibody***, or a fragment thereof (e.g., Fab or F(ab').sub.2) can be used. The term Alabeled@, with regard to the probe or ***antibody***, is intended to encompass direct labeling of the probe or ***antibody*** by coupling (i.e., physically linking) a detectable substance to the probe or ***antibody***, as well as indirect labeling of the probe or ***antibody*** by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary ***antibody*** using a fluorescently labeled secondary ***antibody*** and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled

streptavidin. The term . . . hybridizations. Furthermore, in vivo techniques for detection of a polypeptide of the invention include introducing into a subject a labeled ***antibody*** directed against the polypeptide. For example, the ***antibody*** can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging. . .

DETD . . . in a biological sample and means for determining the amount of the polypeptide or mRNA in the sample (e.g., an ***antibody*** which binds the polypeptide or an oligonucleotide probe which binds to DNA or mRNA encoding the polypeptide). Kits can also. . .

DETD [0235] For ***antibody*** -based kits, the kit can comprise, for example: (1) a first ***antibody*** (e.g., attached to a solid support) which binds to a polypeptide of the invention; and, optionally, (2) a second, different ***antibody*** which binds to either the polypeptide or the first ***antibody*** and is conjugated to a detectable agent.

DETD . . . described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or ***antibody*** reagent described herein, which may be conveniently used, e.g., in clinical settings to diagnose patients exhibiting symptoms or family history. . .

DETD . . . to, e.g., infection, toxins, or drugs), inflammations (e.g., bacterial and viral meningitis, encephalitis, and cerebral toxoplasmosis), cerebrovascular diseases (e.g., hypoxia, ***ischemia***, and infarction, intracranial hemorrhage and vascular malformations, and hypertensive encephalopathy), and tumors (e.g., neuroglial tumors, neuronal tumors, tumors of pineal. . .

DETD . . . system. Consequently, Tango-74 and Tango-76 polypeptides, nucleic acids, and modulators thereof can be used to treat cardiovascular disorders, such as ***ischemic*** heart disease (e.g., angina pectoris, myocardial infarction, and chronic ***ischemic*** heart disease), hypertensive heart disease, pulmonary heart disease, valvular heart disease (e.g., rheumatic fever and rheumatic heart disease, endocarditis, mitral. . .

DETD . . . the heart. Consequently, Tango-74 and Tango-76 nucleic acids, proteins, and modulators thereof can be used to treat heart disorders, e.g., ***ischemic*** heart disease, atherosclerosis, hypertension, angina pectoris, Hypertrophic Cardiomyopathy, and congenital heart disease.

DETD . . . intestines. Consequently, Tango-73 and Tango-74 polypeptides, nucleic acids, and modulators thereof can be used to treat intestinal disorders, such as ***ischemic*** bowel disease, infective enterocolitis, Crohn's disease, benign tumors, malignant tumors (e.g., argentaffinomas, lymphomas, adenocarcinomas, and sarcomas), malabsorption syndromes (e.g., celiac. . .

DETD . . . such as congenital anomalies (e.g., megacolon and imperforate anus), idiopathic disorders (e.g., diverticular disease and melanosis coli), vascular lesions (e.g., ***ischemic*** colitis, hemorrhoids, angiodysplasia), inflammatory diseases (e.g., colitis (e.g., idiopathic ulcerative colitis, pseudomembranous colitis), and lymphopathia venereum), and tumors (e.g., hyperplastic. . .

DETD . . . the biological activities of the polypeptide of the invention. Examples of such inhibitory agents include antisense nucleic acid molecules and ***antibodies***. These modulatory methods can be performed in vitro (e.g., by culturing the cell with the agent) or,

alternatively, in vivo. . .

DETD . . . ascorbic acid, phospholipids, proteins (for example, serum albumin), EDTA, sodium chloride, liposomes, mannitol, sorbitol, and glycerol. The nucleic acids, polypeptides, ***antibodies***, or modulatory compounds of the invention can be administered by any standard route of administration. For example, administration can be .

DETD . . . disorder described herein can be simultaneously or sequentially administered both a cytotoxic agent which selectively kills aberrant cells and an ***antibody*** (e.g., an ***antibody*** of the invention) which can, in one embodiment, be conjugated or linked with a therapeutic agent, a cytotoxic agent, an. . .

CLM What is claimed is:

11. An ***antibody*** which selectively binds with the polypeptide of claim 8.

14. The method of claim 13, wherein the compound which binds with the polypeptide is an ***antibody***.

23. An ***antibody*** substance which selectively binds with the polypeptide of claim 8.

24. A method of making an ***antibody*** substance which selectively binds with the polypeptide of claim 8, the method comprising providing the polypeptide to an immunocompetent vertebrate and thereafter harvesting from the vertebrate blood or serum comprising the ***antibody*** substance.

25. A method of making an ***antibody*** substance which selectively binds with the polypeptide of claim 8, the method comprising contacting the polypeptide with a plurality of particles which individually comprise an ***antibody*** substance and a nucleic acid encoding the ***antibody*** substance, segregating a particle which selectively binds with the polypeptide, and expressing the ***antibody*** substance from the nucleic acid of the segregated particle.

L9 ANSWER 4 OF 9 USPATFULL on STN

AN 2003:152781 USPATFULL

TI Novel genes encoding proteins having prognostic, diagnostic, preventive, therapeutic and other uses

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PA Millennium Pharmaceuticals, Inc. (U.S. corporation)

PI US 2003104447 A1 20030605

AI US 2002-269353 A1 20021011 (10)

RLI Continuation of Ser. No. US 2001-790264, filed on 21 Feb 2001, ABANDONED

Continuation-in-part of Ser. No. US 1999-298531, filed on 23 Apr 1999,

ABANDONED Continuation-in-part of Ser. No. US 1998-65661, filed on 23

Apr 1998, ABANDONED

DT Utility

FS APPLICATION

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CLMN Number of Claims: 64

ECL Exemplary Claim: 1

DRWN 45 Drawing Page(s)

LN.CNT 7262

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention provides isolated nucleic acids encoding a variety of proteins having diagnostic, preventive, therapeutic, and other uses. These nucleic and proteins are useful for diagnosis, prevention, and therapy of a number of human and other animal disorders. The invention also provides antisense nucleic acid molecules, expression vectors containing the nucleic acid molecules of the invention, host cells into which the expression vectors have been introduced, and non-human transgenic animals in which a nucleic acid molecule of the invention has been introduced or disrupted. The invention still further provides isolated polypeptides, fusion polypeptides, antigenic peptides and ***antibodies***. Diagnostic, screening, and therapeutic methods using compositions of the invention are also provided. The nucleic acids and polypeptides of the present invention are useful as modulating agents in regulating a variety of cellular processes.

IN ***McCarthy, Sean A.***, San Diego, CA, UNITED STATES

AB . . . of the invention has been introduced or disrupted. The invention still further provides isolated polypeptides, fusion polypeptides, antigenic peptides and ***antibodies***. Diagnostic, screening, and therapeutic methods using compositions of the invention are also provided. The nucleic acids and polypeptides of the . . .

SUMM . . . a polypeptides not part of the invention (e.g., heterologous amino acid sequences) to form fusion proteins. The invention further features ***antibodies*** that specifically bind to polypeptides of the invention, such as monoclonal or polyclonal ***antibodies***. In addition, the polypeptides of the invention or biologically active portions thereof can be incorporated into pharmaceutical compositions, which optionally. . .

SUMM . . . or expression such that this activity or expression in the cell is modulated. In one embodiment, the agent is an ***antibody*** that specifically binds to polypeptide of the invention. In another embodiment, the agent modulates expression of nucleic acid or polypeptide. . .

DETD . . . neutralizing proteinases produced by bone marrow accessory cells. Accordingly, TANGO-175 and WDNM-2 polypeptides and nucleic acid molecules, anti-TANGO-175 and WDNM-2 ***antibodies***, and modulators of TANGO-175 and WDNM-2 expression or activity may be useful in the treatment and diagnosis of cancer, inflammation,. . .

DETD . . . TANGO-175 is consistent with a role in coagulation. Accordingly, TANGO-175 and WDNM-2 polypeptides and nucleic acid molecules, anti-TANGO-175 and anti-WDNM-2 ***antibodies***, and modulators of TANGO-175 or WDNM-2 expression or activity may be useful in the treatment and diagnosis of cancer, inflammation,. . .

DETD . . . receptors or antigens expressed on a selected cell surface, e.g., by linking the antisense nucleic acid molecules to peptides or ***antibodies*** which bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using.

DETD [0209] Isolated TANGO-139, 125, 110, 175 or WDNM-2 Proteins and Anti-TANGO-139, 125, 110, 175, or WDNM-2 ***Antibodies***

DETD . . . the invention, and biologically active portions thereof, as

well as polypeptide fragments suitable for use as immunogens to raise anti-polypeptides-of-the-invention ***antibodies***. In one embodiment, native polypeptides of the invention can be isolated from cells or tissue sources by an appropriate purification. . .

DETD . . . immunoglobulin and polypeptides of the invention that are part of the invention can be used as immunogens to produce anti-polypeptide-of-the-invention ***antibodies*** in a subject, to purify ligands of polypeptides of the invention and in screening assays to identify molecules which inhibit. . .

DETD . . . An isolated polypeptide of the invention, or a portion or fragment thereof, can be used as an immunogen to generate ***antibodies*** that bind a polypeptide of the invention using standard techniques for polyclonal and monoclonal ***antibody*** preparation. The full-length polypeptide of the invention can be used or, alternatively, the invention provides antigenic peptide fragments of polypeptides. . . (e.g., that shown in SEQ ID NO:54) and encompasses an epitope of a polypeptide of the invention such that an ***antibody*** raised against the peptide forms a specific immune complex with a polypeptide of the invention.

DETD [0235] A polypeptide-of-the-invention immunogen typically is used to prepare ***antibodies*** by immunizing a suitable subject, (e.g., rabbit, goat, mouse or other mammal) with the immunogen. An appropriate immunogenic preparation can. . . incomplete adjuvant, or similar immunostimulatory agent. Immunization of a suitable subject with an immunogenic polypeptide-of-the-invention preparation induces a polyclonal anti-polypeptide-of-the-invention ***antibody*** response.

DETD [0236] Accordingly, another aspect of the invention pertains to anti-polypeptide-of-the-invention ***antibodies***. The term " ***antibody*** " as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen. . . Examples of immunologically active portions of immunoglobulin molecules include F(ab) and F(ab').sub.2 fragments which can be generated by treating the ***antibody*** with an enzyme such as pepsin or papein, especially. The invention provides polyclonal and monoclonal ***antibodies*** that bind a polypeptide of the invention. The term "monoclonal ***antibody*** " or "monoclonal ***antibody*** composition", as used herein, refers to a population of ***antibody*** molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of a polypeptide of the invention. A monoclonal ***antibody*** composition thus typically displays a single binding affinity for a particular polypeptide of the invention with which it immunoreacts.

DETD [0237] Polyclonal anti-polypeptide-of-the-invention ***antibodies*** can be prepared as described above by immunizing a suitable subject with a polypeptide-of-the-invention immunogen. The anti-polypeptide-of-the-invention ***antibody*** titer in the immunized subject can be monitored over time by standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using immobilized polypeptide of the invention. If desired, the ***antibody*** molecules directed against a polypeptide of the invention can be isolated from the mammal (e.g., from the blood) and further. . . such as protein A chromatography to obtain the IgG fraction. At an appropriate time after immunization, e.g., when the anti-polypeptide-of-the-invention ***antibody***

titers are highest, ***antibody*** -producing cells can be obtained from the subject and used to prepare monoclonal ***antibodies*** by standard techniques, such as the hybridoma technique originally described by Kohler and Milstein (1975) *Nature* 256:495-497, the human B cell hybridoma technique (Kozbor et al. (1983) *Immunol. Today* 4:72), the EBV-hybridoma technique (Cole et al. (1985), *Monoclonal ***Antibodies*** and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96) or trioma techniques. The technology for producing various ***antibodies***, monoclonal ***antibody*** hybridomas is well known (see generally *Current Protocols in Immunology* (1994) Coligan et al. (eds.) John Wiley & Sons, Inc., . . . described above, and the culture supernatants of the resulting hybridoma cells are screened to identify a hybridoma producing a monoclonal ***antibody*** that binds a polypeptide of the invention.

DETD . . . protocols used for fusing lymphocytes and immortalized cell lines can be applied for the purpose of generating an anti-polypeptide-of-the-invention monoclonal ***antibody*** (see, e.g., *Current Protocols in Immunology*, supra; Galfre et al. (1977) *Nature* 266:55052; R. H. Kenneth, in *Monoclonal ***Antibodies*** : A New Dimension In Biological Analyses*, Plenum Publishing Corp., New York, N.Y. (1980); and Lerner (1981) *Yale J. Biol. Med.*, . . . unproductively fused myeloma cells (unfused splenocytes die after several days because they are not transformed). Hybridoma cells producing a monoclonal ***antibody*** of the invention are detected by screening the hybridoma culture supernatants for ***antibodies*** that bind a polypeptide of the invention, e.g., using a standard ELISA assay.

DETD [0239] Alternative to preparing monoclonal ***antibody*** -secreting hybridomas, a monoclonal anti-polypeptide-of-the-invention ***antibody*** can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (e.g., an ***antibody*** phage display library) with a polypeptide of the invention to thereby isolate immunoglobulin library members that bind a polypeptide of the invention. Kits for generating and screening phage display libraries are commercially available (e.g., the Pharmacia Recombinant Phage ***Antibody*** System, Catalog No. 27-9400-01; and the Stratagene SurfZAP Phage Display Kit, Catalog No. 240612). Additionally, examples of methods and reagents particularly amenable for use in generating and screening ***antibody*** display library can be found in, for example, U.S. Pat. No. 5,223,409; PCT Publication No. WO 92/18619; PCT Publication No. . . . Publication No. WO 92/09690; PCT Publication No. WO 90/02809; Fuchs et al. (1991) *Bio/Technology* 9:1370-1372; Hay et al. (1992) *Hum. ***Antibod*** . Hybridomas* 3:81-85; Huse et al. (1989) *Science* 246:1275-1281; Griffiths et al. (1993) *EMBO J.* 12:725-734.

DETD [0240] Additionally, recombinant anti-polypeptide-of-the-invention ***antibodies***, such as chimeric and humanized monoclonal ***antibodies***, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the invention. Such chimeric and humanized monoclonal ***antibodies*** can be produced by recombinant DNA techniques known in the art, for example using methods described in PCT Publication No..

DETD [0241] Completely human ***antibodies*** are particularly desirable for therapeutic treatment of human patients. Such ***antibodies***

can be produced using transgenic mice which are incapable of expressing endogenous immunoglobulin heavy and light chains genes, but which. . . in the normal fashion with a selected antigen, e.g., all or a portion of a polypeptide of the invention. Monoclonal ***antibodies*** directed against the antigen can be obtained using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice. . . switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA and IgE ***antibodies***. For an overview of this technology for producing human ***antibodies***, see Lonberg and Huszar (1995, Int. Rev. Immunol. 13:65-93). For a detailed discussion of this technology for producing human ***antibodies*** and human monoclonal ***antibodies*** and protocols for producing such ***antibodies***, see, e.g., U.S. Pat. No. 5,625,126; U.S. Pat. No. 5,633,425; U.S. Pat. No. 5,569,825; U.S. Pat. No. 5,661,016; and U.S. Pat. No. 5,545,806. In addition, companies such as Abgenix, Inc. (Freemont, Calif.), can be engaged to provide human ***antibodies*** directed against a selected antigen using technology similar to that described above.

DETD [0242] Completely human ***antibodies*** which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal ***antibody***, e.g., a murine ***antibody***, is used to guide the selection of a completely human ***antibody*** recognizing the same epitope.

DETD [0243] First, a non-human monoclonal ***antibody*** which binds a selected antigen (epitope), e.g., an ***antibody*** which inhibits activity, is identified. The heavy chain and the light chain of the non-human ***antibody*** are cloned and used to create phage display Fab fragments. For example, the heavy chain gene can be cloned into. . . to phage is used to infect the bacteria which express the non-human heavy chain. The resulting progeny phage display hybrid ***antibodies*** (human light chain/non-human heavy chain). The selected antigen is used in a panning screen to select phage which bind the. . . light chains are infected with a repertoire of human heavy chains fused to phage. The resulting progeny phage display human ***antibodies*** (human light chain/human heavy chain).

DETD . . . panning screen to select phage which bind the selected antigen. The phage selected in this step display a completely human ***antibody*** which recognizes the same epitope recognized by the original selected, non-human monoclonal ***antibody***. The genes encoding both the heavy and light chains are readily isolated and can be further manipulated for production of human ***antibody***. This technology is described by Jespers et al. (1994, Bio/technology 12:899-903).

DETD [0245] An anti-polypeptide-of-the-invention ***antibody*** (e.g., monoclonal ***antibody***) can be used to isolate a polypeptide of the invention by standard techniques, such as affinity chromatography or immunoprecipitation. An anti-polypeptide-of-the-invention ***antibody*** can facilitate the purification of natural polypeptide of the invention from cells and of recombinantly produced polypeptide of the invention expressed in host cells. Moreover, an anti-polypeptide-of-the-invention ***antibody*** can be used to detect polypeptide of the invention (e.g., in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the polypeptide of the invention. Anti-polypeptide-of-the-invention ***antibodies***

can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, e.g., to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the ***antibody*** to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and. . .

DETD [0246] An ***antibody*** (or fragment thereof) can be conjugated to a therapeutic moiety such as a cytotoxin, a therapeutic agent, or a radioactive. . .

DETD [0247] Conjugated ***antibodies*** of the invention can be used for modifying a given biological response, the drug moiety not being limited to classical. . .

DETD [0248] Techniques for conjugating a therapeutic moiety to an ***antibody*** are well known (see, e.g., Arnon et al., 1985, "Monoclonal ***Antibodies*** For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal ***Antibodies*** And Cancer Therapy, Reisfeld et al., Eds., Alan R. Liss, Inc. pp. 243-256; Hellstrom et al., 1987, " ***Antibodies*** For Drug Delivery", in Controlled Drug Delivery, 2nd ed., Robinson et al., Eds., Marcel Dekker, Inc., pp. 623-653; Thorpe, 1985, " ***Antibody*** Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal ***Antibodies*** '84: Biological And Clinical Applications, Pinchera et al., Eds., pp. 475-506; "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled ***Antibody*** In Cancer Therapy", in Monoclonal ***Antibodies*** For Cancer Detection And Therapy, Baldwin et al., Eds., Academic Press, pp. 303-316, 1985; and Thorpe et al., 1982, Immunol. Rev., 62:119-158). Alternatively, an ***antibody*** can be conjugated to a second ***antibody*** to form an ***antibody*** heteroconjugate as described by Segal in U.S. Pat. No. 4,676,980.

DETD [0272] The nucleic acids of the invention, polypeptides of the invention, and anti-polypeptide-of-the-invention ***antibodies*** (also referred to herein as "active compounds") of the invention can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the nucleic acid molecule, protein, or ***antibody*** and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all. . .

DETD . . . kilogram to about 500 milligrams per kilogram, or about 1 milligram per kilogram to about 50 milligrams per kilogram). For ***antibodies***, examples of dosages are from about 0.1 milligram per kilogram to 100 milligrams per kilogram of body weight (generally 10 milligrams per kilogram to 20 milligrams per kilogram). If the ***antibody*** is to act in the brain, a dosage of 50 milligrams per kilogram to 100 milligrams per kilogram is usually. . .

DETD [0278] Sterile injectable solutions can be prepared by incorporating the active compound (e.g., a polypeptide of the invention or anti-polypeptide-of-the-invention ***antibody***) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed. . .

DETD . . . be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal ***antibodies*** to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to. . .

DETD [0285] Generally, partially human ***antibodies*** and fully human ***antibodies*** have a longer half-life within the human body than other ***antibodies***. Accordingly, lower dosages and less frequent administration is often possible. Modifications such as lipidation can be used to stabilize ***antibodies*** and to enhance uptake and tissue penetration (e.g., into the brain). A method for lipidation of ***antibodies*** is described by Cruikshank et al. ((1997) J. Acquired Immune Deficiency Syndromes and Human Retrovirology 14:193).

DETD . . . disorder described herein can be simultaneously or sequentially administered both a cytotoxic agent which selectively kills aberrant cells and an ***antibody*** (e.g., an ***antibody*** of the invention) which can, in one embodiment, be conjugated or linked with a therapeutic agent, a cytotoxic agent, an. . .

DETD [0290] The nucleic acid molecules, proteins, protein homologues, and ***antibodies*** described herein can be used in one or more of the following methods: a) screening assays; b) detection assays (e.g., . . . the invention which have decreased or aberrant activity compared to wild type polypeptide of the invention. In addition, the anti-polypeptide-of-the-invention ***antibodies*** of the invention can be used to detect and isolate polypeptides of the invention and modulate activity of polypeptides of. . .

DETD . . . (e.g., biotinylation kit, Pierce Chemicals; Rockford, Ill.), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, ***antibodies*** reactive with polypeptide of the invention or target molecules but which do not interfere with binding of the polypeptide of. . . derivatized to the wells of the plate, and unbound target or polypeptide of the invention trapped in the wells by ***antibody*** conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using ***antibodies*** reactive with the polypeptide of the invention or target molecule, as well as enzyme-linked assays which rely on detecting an. . .

DETD [0341] A preferred agent for detecting polypeptide of the invention is an ***antibody*** capable of binding to polypeptide of the invention, preferably an ***antibody*** with a detectable label. ***Antibodies*** can be polyclonal, or more preferably, monoclonal. An intact ***antibody***, or a fragment thereof (e.g., Fab or F(ab').sub.2) can be used. The term "labeled", with regard to the probe or ***antibody***, is intended to encompass direct labeling of the probe or ***antibody*** by coupling (i.e., physically linking) a detectable substance to the probe or ***antibody***, as well as indirect labeling of the probe or ***antibody*** by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary ***antibody*** using a fluorescently labeled secondary ***antibody*** and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin. The term. . . hybridizations. Furthermore, in vivo techniques for detection of polypeptide of the invention include introducing into a subject a labeled anti-polypeptide-of-the-invention ***antibody***. For example, the ***antibody*** can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging. . .

DETD . . . sample and means for determining the amount of polypeptide or mRNA of the invention in the sample (e.g., an anti-polypeptide-of-the-

invention ***antibody*** or an oligonucleotide probe which binds to DNA encoding polypeptide of the invention (e.g., SEQ ID NO:1, SEQ ID NO:3, . . .

DETD [0345] For ***antibody*** -based kits, the kit may comprise, for example: (1) a first ***antibody*** (e.g., attached to a solid support) which binds to polypeptide of the invention; and, optionally, (2) a second, different ***antibody*** which binds to polypeptide of the invention or the first ***antibody*** and is conjugated to a detectable agent.

DETD . . . described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or ***antibody*** reagent described herein, which may be conveniently used, e.g., in clinical settings to diagnose patients exhibiting symptoms or family history. . .

DETD . . . polypeptide of the invention. Examples of such inhibitory agents include antisense molecules of nucleic acids of the invention and anti-polypeptide-of-the-invention ***antibodies***. These modulatory methods can be performed in vitro (e.g., by culturing the cell with the agent) or, alternatively, in vivo. . .

DETD . . . heart, TANGO 125, 110, and 175 nucleic acids, proteins, and modulators thereof can be used to treat heart disorders, e.g., ***ischemic*** heart disease, atherosclerosis, hypertension, angina pectoris, Hypertrophic Cardiomyopathy, and congenital heart disease.

DETD . . . TANGO 125, 110, and 175 polypeptides, nucleic acids, and modulators thereof can be used to treat cardiovascular disorders, such as ***ischemic*** heart disease (e.g., angina pectoris, myocardial infarction, and chronic ***ischemic*** heart disease), hypertensive heart disease, pulmonary heart disease, valvular heart disease (e.g., rheumatic fever and rheumatic heart disease, endocarditis, mitral. . .

DETD . . . to, e.g., infection, toxins, or drugs), inflammations (e.g., bacterial and viral meningitis, encephalitis, and cerebral toxoplasmosis), cerebrovascular diseases (e.g., hypoxia, ***ischemia***, and infarction, intracranial hemorrhage and vascular malformations, and hypertensive encephalopathy), and tumors (e.g., neuroglial tumors, neuronal tumors, tumors of pineal. . .

DETD . . . In another example, TANGO 125 polypeptides, nucleic acids, and modulators thereof can be used to treat intestinal disorders, such as ***ischemic*** bowel disease, infective enterocolitis, Crohn's disease, benign tumors, malignant tumors (e.g., argentaffinomas, lymphomas, adenocarcinomas, and sarcomas), malabsorption syndromes (e.g., celiac. . .

DETD . . . such as congenital anomalies (e.g., megacolon and imperforate anus), idiopathic disorders (e.g., diverticular disease and melanosis coli), vascular lesions (e.g., ***ischemic*** colitis, hemorrhoids, angiodysplasia), inflammatory diseases (e.g., colitis (e.g., idiopathic ulcerative colitis, pseudomembranous colitis), and lymphopathia venereum), Crohn's disease, and tumors. . .

DETD . . . according to the manufacturer's instructions. Conditioned medium samples are analyzed by SDS-PAGE followed by Western blot using anti-human alkaline phosphatase ***antibodies*** diluted 1:250 (Genzyme Corp., Cambridge Mass.) and detected by chemiluminescence.

CLM What is claimed is:

11. An ***antibody*** which selectively binds with the polypeptide of claim 8.

14. The method of claim 13, wherein the compound which binds with the polypeptide is an ***antibody*** .

23. An ***antibody*** substance which selectively binds with the polypeptide of claim 8.

24. A method of making an ***antibody*** substance which selectively binds with the polypeptide of claim 8, the method comprising providing the polypeptide to an immunocompetent vertebrate and thereafter harvesting from the vertebrate blood or serum comprising the ***antibody*** substance.

25. A method of making an ***antibody*** substance which selectively binds with the polypeptide of claim 8, the method comprising contacting the polypeptide with a plurality of particles which individually comprise an ***antibody*** substance and a nucleic acid encoding the ***antibody*** substance, segregating a particle which selectively binds with the polypeptide, and expressing the ***antibody*** substance from the nucleic acid of the segregated particle.

L9 ANSWER 5 OF 9 USPATFULL on STN

AN 2003:120196 USPATFULL

TI Novel EDIRF, MTR-1, LSP-1, TAP-1, and PA-I molecules and uses therefor

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RLI Continuation-in-part of Ser. No. US 1997-941354, filed on 30 Sep 1997, ABANDONED Continuation-in-part of Ser. No. US 1998-10674, filed on 22 Jan 1998, ABANDONED Continuation-in-part of Ser. No. US 1998-14347, filed on 27 Jan 1998, ABANDONED Continuation-in-part of Ser. No. US 1999-474151, filed on 28 Dec 1999, GRANTED, Pat. No. US 6267003 Continuation of Ser. No. US 1998-4206, filed on 8 Jan 1998, ABANDONED Continuation of Ser. No. US 2000-483414, filed on 14 Jan 2000, ABANDONED Continuation of Ser. No. US 1998-213571, filed on 18 Dec 1998, ABANDONED Continuation-in-part of Ser. No. US 1997-994890, filed on 19 Dec 1997, ABANDONED

PRAI US 1997-61149P 19971006 (60)

US 1997-61159P 19971006 (60)

US 1997-61143P 19971006 (60)

DT Utility

FS APPLICATION

LREP Intellectual Property Group, MILLENNIUM PHARMACEUTICALS, INC., 75 Sidney Street, Cambridge, MA, 02139

CLMN Number of Claims: 46

ECL Exemplary Claim: 1

DRWN 4 Drawing Page(s)

LN.CNT 8025

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Novel EDIRF, MTR-1, LSP-1, TAP-1, and PA-I polypeptides, proteins, and nucleic acid molecules are disclosed. In addition to isolated,

full-length EDIRF, MTR-1, LSP-1, TAP-1, and PA-I proteins, the invention further provides isolated EDIRF, MTR-1, LSP-1, TAP-1, and PA-I fusion proteins, antigenic peptides and anti-EDIRF, MTR-1, LSP-1, TAP-1, and PA-I ***antibodies***. The invention also provides EDIRF, MTR-1, LSP-1, TAP-1, and PA-I nucleic acid molecules, recombinant expression vectors containing a nucleic acid molecule of the invention, host cells into which the expression vectors have been introduced and non-human transgenic animals in which an EDIRF, MTR-1, LSP-1, TAP-1, and PA-I gene has been introduced or disrupted. Diagnostic, screening and therapeutic methods utilizing compositions of the invention are also provided.

IN ***McCarthy, Sean A.***, San Diego, CA, UNITED STATES

AB . . . further provides isolated EDIRF, MTR-1, LSP-1, TAP-1, and PA-I fusion proteins, antigenic peptides and anti-EDIRF, MTR-1, LSP-1, TAP-1, and PA-I ***antibodies***. The invention also provides EDIRF, MTR-1, LSP-1, TAP-1, and PA-I nucleic acid molecules, recombinant expression vectors containing a nucleic acid. . .

SUMM . . . domain. Such proteins function in a variety of immune cell functions ranging from immune cell development and differentiation, antigen recognition, ***antibody*** production, cellular signal transduction, and cellular homing of immune responsive cells from the circulation to sites of increased antigen concentration.

SUMM . . . non-MTR-1, non-LSP-1, non-TAP-1, or non-PA-I polypeptide to form EDIRF, MTR-1, LSP-1, TAP-1, and PA-I fusion proteins. The invention further features ***antibodies*** that specifically bind EDIRF, MTR-1, LSP-1, TAP-1, or PA-I proteins, such as monoclonal or polyclonal ***antibodies***. In addition, the EDIRF, MTR-1, LSP-1, TAP-1, and PA-I proteins or biologically active portions thereof can be incorporated into pharmaceutical. . .

SUMM . . . In another embodiment, the agent stimulates EDIRF, MTR-1, LSP-1, TAP-1, or PA-I activity. In one embodiment, the agent is an ***antibody*** that specifically binds to EDIRF, MTR-1, LSP-1, TAP-1, or PA-I protein. In another embodiment, the agent modulates expression of EDIRF,. . .

DETD . . . receptors or antigens expressed on a selected cell surface, e.g., by linking the antisense nucleic acid molecules to peptides or ***antibodies*** which bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using.

DETD [0181] II. Isolated Proteins and ***Antibodies***

DETD . . . thereof, as well as polypeptide fragments suitable for use as immunogens to raise anti- EDIRF, MTR-1, LSP-1, TAP-1, and PA-I ***antibodies***. In one embodiment, native EDIRF, MTR-1, LSP-1, TAP-1, and PA-I proteins can be isolated from cells or tissue sources by. . .

DETD . . . or PA-I-immunoglobulin fusion proteins of the invention can be used as immunogens to produce anti-EDIRF, MTR-1, LSP-1, TAP-1, or PA-I ***antibodies*** in a subject, to purify EDIRF, MTR-1, LSP-1, TAP-1, or PA-I receptors and in screening assays to identify molecules which.

DETD . . . MTR-1, LSP-1, TAP-1, or PA-I protein, or a portion or fragment thereof, can be used as an immunogen to generate ***antibodies*** that bind EDIRF, MTR-1, LSP-1, TAP-1, or PA-I using standard techniques for polyclonal and monoclonal ***antibody*** preparation. The full-length EDIRF, MTR-1, LSP-1, TAP-1, or PA-I protein can be used or, alternatively, the invention provides antigenic peptide. . . 6, 35,

44, 50, or 54 and encompasses an epitope of EDIRF, MTR-1, LSP-1, TAP-1, or PA-I such that an ***antibody*** raised against the peptide forms a specific immune complex with EDIRF, MTR-1, LSP-1, TAP-1, or PA-I. Preferably, the antigenic peptide. . .

DETD [0209] A EDIRF, MTR-1, LSP-1, TAP-1, or PA-I immunogen typically is used to prepare ***antibodies*** by immunizing a suitable subject, (e.g., rabbit, goat, mouse or other mammal) with the immunogen. An appropriate immunogenic preparation can. . . subject with an immunogenic EDIRF, MTR-1, LSP-1, TAP-1, or PA-I preparation induces a polyclonal anti-EDIRF, MTR-1, LSP-1, TAP-1, or PA-I ***antibody*** response.

DETD [0210] Accordingly, another aspect of the invention pertains to anti-EDIRF, MTR-1, LSP-1, TAP-1, or PA-I ***antibodies***. The term " ***antibody*** " as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen. . . Examples of immunologically active portions of immunoglobulin molecules include F(ab) and F(ab').sub.2 fragments which can be generated by treating the ***antibody*** with an enzyme such as pepsin or papain, respectively. The invention provides polyclonal and monoclonal ***antibodies*** that bind EDIRF, MTR-1, LSP-1, TAP-1, or PA-I. The term "monoclonal ***antibody*** " or "monoclonal ***antibody*** composition", as used herein, refers to a population of ***antibody*** molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of EDIRF, MTR-1, LSP-1, TAP-1, or PA-I. A monoclonal ***antibody*** composition thus typically displays a single binding affinity for a particular EDIRF, MTR-1, LSP-1, TAP-1, or PA-I protein with which. . .

DETD [0211] Polyclonal anti-EDIRF, MTR-1, LSP-1, TAP-1, or PA-I ***antibodies*** can be prepared as described above by immunizing a suitable subject with an EDIRF, MTR-1, LSP-1, TAP-1, or PA-I immunogen. The anti-EDIRF, MTR-1, LSP-1, TAP-1, or PA-I ***antibody*** titer in the immunized subject can be monitored over time by standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using immobilized EDIRF, MTR-1, LSP-1, TAP-1, or PA-I. If desired, the ***antibody*** molecules directed against EDIRF, MTR-1, LSP-1, TAP-1, or PA-I can be isolated from the mammal (e.g., from the blood) and. . . to obtain the IgG fraction. At an appropriate time after immunization, e.g., when the anti-EDIRF, MTR-1, LSP-1, TAP-1, or PA-I ***antibody*** titers are highest, ***antibody*** -producing cells can be obtained from the subject and used to prepare monoclonal ***antibodies*** by standard techniques, such as the hybridoma technique originally described by Kohler and Milstein (1975) Nature 256:495-497) (see also, Brown. . . human B cell hybridoma technique (Kozbor et al. (1983) Immunol Today 4:72), the EBV-hybridoma technique (Cole et al. (1985), Monoclonal ***Antibodies*** and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96) or trioma techniques. The technology for producing monoclonal ***antibody*** hybridomas is well known (see generally R. H. Kenneth, in Monoclonal ***Antibodies*** : A New Dimension In Biological Analyses, Plenum Publishing Corp., New York, N.Y. (1980); E. A. Lerner (1981) Yale J. Biol.. . . described above, and the culture supernatants of the resulting hybridoma cells are screened to identify a hybridoma producing a monoclonal ***antibody*** that binds EDIRF, MTR-1, LSP-1, TAP-1, or PA-I.

DETD . . . and immortalized cell lines can be applied for the purpose of generating an anti-EDIRF, MTR-1, LSP-1, TAP-1, or PA-I monoclonal

antibody (see, e.g., G. Galfre et al. (1977) Nature 266:55052; Gefter et al. Somatic Cell Genet., cited supra; Lerner, Yale J. Biol. Med., cited supra; Kenneth, Monoclonal ***Antibodies***, cited supra). Moreover, the ordinarily skilled worker will appreciate that there are many variations of such methods which also would. . . unproductively fused myeloma cells (unfused splenocytes die after several days because they are not transformed). Hybridoma cells producing a monoclonal ***antibody*** of the invention are detected by screening the hybridoma culture supernatants for ***antibodies*** that bind EDIRF, MTR-1, LSP-1, TAP-1, or PA-I, e.g., using a standard ELISA assay.

DETD [0213] Alternative to preparing monoclonal ***antibody*** -secreting hybridomas, a monoclonal anti-EDIRF, MTR-1, LSP-1, TAP-1, or PA-I ***antibody*** can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (e.g., an ***antibody*** phage display library) with EDIRF, MTR-1, LSP-1, TAP-1, or PA-I to thereby isolate immunoglobulin library members that bind EDIRF, MTR-1, . . . LSP-1, TAP-1, or PA-I. Kits for generating and screening phage display libraries are commercially available (e.g., the Pharmacia Recombinant Phage ***Antibody*** System, Catalog No. 27-9400-01; and the Stratagene SurfZAP.TM. Phage Display Kit, Catalog No. 240612). Additionally, examples of methods and reagents particularly amenable for use in generating and screening ***antibody*** display library can be found in, for example, Ladner et al. U.S. Pat. No. 5,223,409; Kang et al. PCT International. . . Ladner et al. PCT International Publication No. WO 90/02809; Fuchs et al. (1991) Bio/Technology 9:1370-1372; Hay et al. (1992) Hum. ***Antibod*** . Hybridomas 3:81-85; Huse et al. (1989) Science 246:1275-1281; Griffiths et al. (1993) EMBO J 12:725-734; Hawkins et al. (1992) J. . .

DETD [0214] Additionally, recombinant anti-EDIRF, MTR-1, LSP-1, TAP-1, or PA-I ***antibodies***, such as chimeric and humanized monoclonal ***antibodies***, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the invention. Such chimeric and humanized monoclonal ***antibodies*** can be produced by recombinant DNA techniques known in the art, for example using methods described in Robinson et al.. .

DETD [0215] Completely human ***antibodies*** are particularly desirable for therapeutic treatment of human patients. Such ***antibodies*** can be produced using transgenic mice which are incapable of expressing endogenous immunoglobulin heavy and light chains genes, but which. . . in the normal fashion with a selected antigen, e.g., all or a portion of a polypeptide of the invention. Monoclonal ***antibodies*** directed against the antigen can be obtained using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice. . . switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, and IgE ***antibodies***. For an overview of this technology for producing human ***antibodies***, see Lonberg and Huszar ((1995) Int. Rev. Immunol. 13:65-93). For a detailed discussion of this technology for producing human ***antibodies*** and human monoclonal ***antibodies*** and protocols for producing such ***antibodies***, see, e.g., U.S. Pat. Nos. 5,625,126; 5,633,425; 5,569,825; 5,661,016; and 5,545,806. In addition, companies such as Abgenix, Inc. (Fremont, Calif.), can be engaged to provide human ***antibodies*** directed

against a selected antigen using technology similar to that described above.

DETD [0216] Completely human ***antibodies*** which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal ***antibody***, e.g., a murine ***antibody***, is used to guide the selection of a completely human ***antibody*** recognizing the same epitope (Jaspers et al. (1994) Biotechnology (NY) 12:899-903).

DETD [0217] An anti-EDIRF, MTR-1, LSP-1, TAP-1, or PA-I ***antibody*** (e.g., monoclonal ***antibody***) can be used to isolate EDIRF, MTR-1, LSP-1, TAP-1, or PA-I by standard techniques, such as affinity chromatography or immunoprecipitation. An anti-EDIRF, MTR-1, LSP-1, TAP-1, or PA-I ***antibody*** can facilitate the purification of natural EDIRF, MTR-1, LSP-1, TAP-1, or PA-I from cells and of recombinantly produced EDIRF expressed in host cells. Moreover, an anti-EDIRF, MTR-1, LSP-1, TAP-1, or PA-I ***antibody*** can be used to detect EDIRF, MTR-1, LSP-1, TAP-1, or PA-I protein (e.g., in a cellular lysate or cell supernatant). . . abundance and pattern of expression of the EDIRF, MTR-1, LSP-1, TAP-1, or PA-I protein. Anti-EDIRF, MTR-1, LSP-1, TAP-1, or PA-I ***antibodies*** can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, e.g., to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (i.e., physically linking) the ***antibody*** to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and. . .

DETD [0218] An ***antibody*** (or fragment thereof) can be conjugated to a therapeutic moiety such as a cytotoxin, a therapeutic agent, or a radioactive. . .

DETD [0219] Conjugated ***antibodies*** of the invention can be used for modifying a given biological response, the drug moiety not being limited to classical. . .

DETD [0220] Techniques for conjugating a therapeutic moiety to an ***antibody*** are well known (see, e.g., Arnon et al. (1985) "Monoclonal ***Antibodies*** For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal ***Antibodies*** And Cancer Therapy, Reisfeld et al., Eds., Alan R. Liss, Inc. pp. 243-256; Hellstrom et al. (1987) " ***Antibodies*** For Drug Delivery ", in Controlled Drug Delivery, 2nd ed., Robinson et al., Eds., Marcel Dekker, Inc., pp. 623-653; Thorpe, 1985 " ***Antibody*** Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal ***Antibodies*** '84: Biological And Clinical Applications, Pinchera et al., Eds., pp. 475-506; "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled ***Antibody*** In Cancer Therapy", in Monoclonal ***Antibodies*** For Cancer Detection And Therapy, Baldwin et al., Eds., Academic Press, pp. 303-316, 1985; and Thorpe et al. (1982) Immunol. Rev. 62:119-158). Alternatively, an ***antibody*** can be conjugated to a second ***antibody*** to form an ***antibody*** heteroconjugate as described by Segal in U.S. Pat. No. 4,676,980.

DETD . . . EDIRF, MTR-1, LSP-1, TAP-1, or PA-I ligand binding (e.g., direct assays or competitive assays described in detail below), to generate ***antibodies*** specific for EDIRF, MTR-1, LSP-1, TAP-1, or PA-I proteins, as examples. In a preferred embodiment, an EDIRF, MTR-1, LSP-1, TAP-1,. . .

DETD . . . or PA-I nucleic acid molecules, EDIRF, MTR-1, LSP-1, TAP-1, or PA-I proteins, and anti- EDIRF, MTR-1, LSP-1, TAP-1, and PA-I ***antibodies*** (also referred to herein as "active compounds") of the invention can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the nucleic acid molecule, protein, or ***antibody*** and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all. . .

DETD . . . kilogram to about 500 milligrams per kilogram, or about 1 milligram per kilogram to about 50 milligrams per kilogram). For ***antibodies***, examples of dosages are from about 0.1 milligram per kilogram to 100 milligrams per kilogram of body weight (generally 10 milligrams per kilogram to 20 milligrams per kilogram). If the ***antibody*** is to act in the brain, a dosage of 50 milligrams per kilogram to 100 milligrams per kilogram is usually. . .

DETD . . . incorporating the active compound (e.g., an EDIRF, MTR-1, LSP-1, TAP-1, or PA-I protein or anti-EDIRF, MTR-1, LSP-1, TAP-1, or PA-I ***antibody***) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed. . .

DETD . . . be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal ***antibodies*** to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to. . .

DETD [0262] The nucleic acid molecules, proteins, protein homologues, and ***antibodies*** described herein can be used in one or more of the following methods: a) screening assays; b) detection assays (e.g., . .

DETD . . . or PA-I ligands or the cognate ligands of other membrane-bound receptors. In addition, the anti-EDIRF, MTR-1, LSP-1, TAP-1, or PA-I ***antibodies*** of the invention can be used to detect and isolate EDIRF, MTR-1, LSP-1, TAP-1, or PA-I proteins and modulate EDIRF,. . .

DETD . . . are expressed in the heart, the polypeptides, nucleic acids, and modulators thereof can be used to treat heart disorders, e.g., ***ischemic*** heart disease, atherosclerosis, hypertension, angina pectoris, Hypertrophic Cardiomyopathy, and congenital heart disease.

DETD . . . MTR-1, LSP-1, TAP-1, or PA-I polypeptides, nucleic acids, and modulators thereof can be used to treat cardiovascular disorders, such as ***ischemic*** heart disease (e.g., angina pectoris, myocardial infarction, and chronic ***ischemic*** heart disease), hypertensive heart disease, pulmonary heart disease, valvular heart disease (e.g., rheumatic fever and rheumatic heart disease, endocarditis, mitral. . .

DETD . . . to, e.g., infection, toxins, or drugs), inflammations (e.g., bacterial and viral meningitis, encephalitis, and cerebral toxoplasmosis), cerebrovascular diseases (e.g., hypoxia, ***ischemia***, and infarction, intracranial hemorrhage and vascular malformations, and hypertensive encephalopathy), and tumors (e.g., neuroglial tumors, neuronal tumors, tumors of pineal. . .

DETD . . . MTR-1, LSP-1, TAP-1, or PA-I polypeptides, nucleic acids, and modulators thereof can be used to treat intestinal disorders, such as ***ischemic*** bowel disease, infective enterocolitis, Crohn's disease, benign tumors, malignant tumors (e.g., argentaffinomas, lymphomas, adenocarcinomas, and sarcomas), malabsorption syndromes

(e.g., celiac. . .

DETD . . . such as congenital anomalies (e.g., megacolon and imperforate anus), idiopathic disorders (e.g., diverticular disease and melanosis coli), vascular lesions (e.g., ***ischemic*** colitis, hemorrhoids, angiodysplasia), inflammatory diseases (e.g., colitis (e.g., idiopathic ulcerative colitis, pseudomembranous colitis), and lymphopathia venereum), Crohn's disease, and tumors. . .

DETD . . . (e.g., biotinylation kit, Pierce Chemicals, Rockford, Ill.), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, ***antibodies*** reactive with EDIRF, MTR-1, LSP-1, TAP-1, or PA-I or target molecules but which do not interfere with binding of the. . . the wells of the plate, and unbound target or EDIRF, MTR-1, LSP-1, TAP-1, or PA-I trapped in the wells by ***antibody*** conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using ***antibodies*** reactive with the EDIRF, MTR-1, LSP-1, TAP-1, or PA-I or target molecule, as well as enzyme-linked assays which rely on. . .

DETD . . . agent, an antisense EDIRF, MTR-1, LSP-1, TAP-1, or PA-I nucleic acid molecule, an EDIRF, MTR-1, LSP-1, TAP-1, or PA-I-specific ***antibody***, or an EDIRF, MTR-1, LSP-1, TAP-1, or PA-I binding partner) can be used in an animal model to determine the. . .

DETD [0347] A preferred agent for detecting EDIRF, MTR-1, LSP-1, TAP-1, or PA-I protein is an ***antibody*** capable of binding to EDIRF, MTR-1, LSP-1, TAP-1, or PA-I protein, preferably an ***antibody*** with a detectable label. ***Antibodies*** can be polyclonal, or more preferably, monoclonal. An intact ***antibody***, or a fragment thereof (e.g., Fab or F(ab').sub.2) can be used. The term "labeled", with regard to the probe or ***antibody***, is intended to encompass direct labeling of the probe or ***antibody*** by coupling (i.e., physically linking) a detectable substance to the probe or ***antibody***, as well as indirect labeling of the probe or ***antibody*** by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary ***antibody*** using a fluorescently labeled secondary ***antibody*** and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin. The term. . . EDIRF, MTR-1, LSP-1, TAP-1, or PA-I protein include introducing into a subject a labeled anti-EDIRF, MTR-1, LSP-1, TAP-1, or PA-I ***antibody***. For example, the ***antibody*** can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging. . .

DETD . . . described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or ***antibody*** reagent described herein, which may be conveniently used, e.g., in clinical settings to diagnose patients exhibiting symptoms or family history. . .

DETD . . . agents include antisense EDIRF, MTR-1, LSP-1, TAP-1, or PA-I nucleic acid molecules and anti- EDIRF, MTR-1, LSP-1, TAP-1, or PA-I ***antibodies***. These modulatory methods can be performed in vitro (e.g., by culturing the cell with the agent) or, alternatively, in vivo.

DETD . . . electroblotted onto a PVDF membrane (Novex) after separation by SDS-PAGE on 4-20% gradient gels and probed with M2 anti-flag polyclonal ***antibody*** diluted 1:500 (Sigma Chemical Co., St. Louis Mo.) .

followed by horseradish peroxidase conjugated sheep anti-mouse
antibody diluted 1:5000 (Amersham Pharmacia Biotech, Inc.,
Piscataway N.J.) developed with chemiluminescent reagents (Renaissance,
DuPont-NEN Research, Boston Mass.) and exposed to. . .

DETD . . . membrane. Conditioned medium samples before and after
concentration were analyzed by SDS-PAGE followed by Western blot using
anti-human alkaline phosphatase ***antibodies*** diluted 1:250
(Genzyme Corp., Cambridge Mass.) and detected by chemiluminescence. A
band at 90 kDa was observed in concentrated supernatants. . .

DETD . . . according to the manufacturer's instructions (Boehringer
Mannheim). Diluted anti-EDIRF I or anti-EDIRF II rabbit antiserum will
be used as primary ***antibody*** .

DETD . . . -20.degree. C., embedded in paraffin and sectioned at 8 um as
above and processed by standard procedures using various primary
antibodies and horseradish peroxidase-conjugated secondary
antibodies for detection.

DETD . . . by radiolabelling (.sup.35S-methionine or .sup.35S-cysteine
available from NEN, Boston, Mass., can be used) and immunoprecipitation
(Harlow, E. and Lane, D. ***Antibodies*** : A Laboratory Manual, Cold
Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1988) using an
HA specific monoclonal ***antibody*** . Briefly, the cells are
labelled for 8 hours with .sup.35S-methionine (or .sup.35S-cysteine).
The culture media are then collected and the. . . 50 mM Tris, pH
7.5). Both the cell lysate and the culture media are precipitated with
an HA specific monoclonal ***antibody*** . Precipitated polypeptides
are then analyzed by SDS-PAGE.

DETD . . . TAP-1, or PA-I polypeptide is detected by radiolabelling and
immunoprecipitation using a EDIRF, MTR-1, LSP-1, TAP-1, or PA-I specific
monoclonal ***antibody*** .

CLM What is claimed is:

11. An ***antibody*** which selectively binds with the polypeptide
of claim 8.

14. The method of claim 13, wherein the compound which binds with the
polypeptide is an ***antibody*** .

23. An ***antibody*** substance which selectively binds with the
polypeptide of claim 8.

24. A method of making an ***antibody*** substance which selectively
binds with the polypeptide of claim 8, the method comprising providing
the polypeptide to an immunocompetent vertebrate and thereafter
harvesting from the vertebrate blood or serum comprising the
antibody substance.

25. A method of making an ***antibody*** substance which selectively
binds with the polypeptide of claim 8, the method comprising contacting
the polypeptide with a plurality of particles which individually
comprise an ***antibody*** substance and a nucleic acid encoding
the ***antibody*** substance, segregating a particle which
selectively binds with the polypeptide, and expressing the
antibody substance from the nucleic acid of the segregated
particle.

L9 ANSWER 6 OF 9 USPATFULL on STN

AN 2003:100085 USPATFULL

TI Compositions and methods for the treatment and diagnosis of immune disorders

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DT Utility

FS APPLICATION

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ECL Exemplary Claim: 1

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CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to methods and compositions for the treatment and diagnosis of immune disorders, especially T helper lymphocyte-related disorders, and also for the treatment of mast cell-related processes and disorders, ***ischemic*** disorders and injuries, including ***ischemic*** renal disorders and injuries. For example, genes which are differentially expressed within and among T helper (TH) cells and TH cell subpopulations, which include, but are not limited to TH0, TH1 and TH2 cell subpopulations are identified. Genes are also identified via the ability of their gene products to interact with gene products involved in the differentiation, maintenance and effector function of such TH cells and TH cell subpopulations. The genes identified can be used diagnostically or as targets for therapeutic intervention. In this regard, the present invention provides methods for the identification and therapeutic use of compounds as treatments of immune disorders, especially TH cell subpopulation-related disorders. Additionally, methods are provided for the diagnostic evaluation and prognosis of TH cell subpopulation-related disorders, for the identification of subjects exhibiting a predisposition to such conditions, for monitoring patients undergoing clinical evaluation for the treatment of such disorders, and for monitoring the efficacy of compounds used in clinical trials. Methods are also provided for the treatment of symptoms associated with mast cell-related processes or disorders and ***ischemic*** disorders and injuries using the genes, gene products and ***antibodies*** of the invention.

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AB . . . diagnosis of immune disorders, especially T helper lymphocyte-related disorders, and also for the treatment of mast cell-related processes and disorders, ***ischemic*** disorders and injuries, including ***ischemic*** renal disorders and injuries. For example, genes which are differentially expressed within and among T

helper (TH) cells and TH. . . in clinical trials. Methods are also provided for the treatment of symptoms associated with mast cell-related processes or disorders and ***ischemic*** disorders and injuries using the genes, gene products and ***antibodies*** of the invention.

SUMM . . . TH cells and TH cell subpopulations. Among the genes identified are ones involved in repair or recovery of tissue from ***ischemic*** disorders or injuries. The genes identified can be used diagnostically or as targets for therapeutic intervention. In this regard, the. . . of compounds as treatments of immune disorders, especially TH cell subpopulation-related disorders. The present invention also provides methods for treating ***ischemic*** disorders or injuries.

Additionally, methods are provided for the diagnostic evaluation and prognosis of TH cell subpopulation-related disorders, for the. . .

SUMM . . . TH cells are involved in both humoral and cell-mediated forms of effector immune responses. With respect to the humoral, or ***antibody***, immune response, ***antibodies*** are produced by B lymphocytes through interactions with TH cells. Specifically, extracellular antigens are endocytosed by antigen-presenting cells (APCs), processed,. . . complex (MHC) molecules to CD4.sup.+ class II MHC-restricted TH cells. These TH cells in turn activate B lymphocytes, resulting in ***antibody*** production.

SUMM . . . cells drive humoral immune responses, which are associated with, for example, defenses against certain helminthic parasites, and are involved in ***antibody*** and allergic responses.

SUMM . . . cell and TH cell-like related disorders. The present invention additionally relates to methods and compositions for treating, ameliorating or modulating ***ischemic*** disorders or injuries or mast cell-related processes or disorders.

SUMM [0026] The present invention also includes the products of such fingerprint, target, and pathway genes, as well as ***antibodies*** to such gene products. Furthermore, the engineering and use of cell- and animal-based models of TH cell subpopulation-related disorders to. . .

SUMM [0029] ***Ischemic*** disorder or injury can be treated via the methods of the invention. " ***Ischemic*** disorder or injury" refers to any disorder or injury to tissues or organs which results from local deficiency of the. . . supply and/or hypoxia are generally produced by a restriction or obstruction of the blood supply to said tissue or organs. ***Ischemic*** disorders or injuries which may be treated by the methods of the present invention include, but are by no means limited to, ***ischemic*** renal disease or injury, or myocardial ***ischemia*** such as angina pectoris. ***Ischemic*** disorders or injuries which may be treated by the methods of the present invention also include damage or injury to. . . intestine, lung, and testes. The methods of the invention may also be used to regulate the extent or degree of ***ischemic*** injury in other tissues, such as tumor tissues including, but not limited to, tumors of the uterus and ovaries. The ***ischemic*** disorders or injuries which may be treated by the methods of the present invention still further include ***ischemic*** injury or damage to transplanted organs which occurs during transplant.

SUMM . . . alternative embodiment of the present invention, the methods a compositions described herein can also be used in the treatment of ***ischemic*** disorders or injuries. For example, presented herein are methods of using the 200 gene, its gene product, and ***antibodies*** thereto to treat or regulate ***ischemic***

disorders and/or injuries. In particular, the genes or gene products of the invention may be administered to an individual so as to ameliorate the symptoms of the ***ischemic*** disorder or injury. Further, compounds, such as specific ***antibodies***, including monoclonal ***antibodies***, which bind specifically to the genes or gene products of the present invention and modulate their expression or activity, may also be administered to an individual suffering from an ***ischemic*** disorder or injury.

SUMM . . . of 10 gene product activity, can be particularly amenable to modulation. For example, natural ligands, derivatives of natural ligands and ***antibodies*** which bind to the 10 gene product can be utilized to reduce the number of induced T cells present by. . .

SUMM . . . product, including, for example, soluble fusion proteins such as Ig-tailed fusion proteins, can be particularly useful for this purpose. Additionally, ***antibodies*** directed against one or more of the extracellular portions of the 10 gene product may either reduce 10 gene product function by, for example, locking ligand binding. Additionally, ***antibodies*** directed against the 10 gene product can, in certain instances, serve to increase the level of 10 gene product activity.

SUMM . . . IgE immune response, including but not limited to the symptoms which accompany atopic conditions such as allergy and/or asthma. IgE-type ***antibodies*** are produced by stimulated B cells which require, at least in part, IL-4 produced by the TH2 cell subpopulation. Therefore,. . .

SUMM . . . one of its gene products is a membrane-bound TH2 cell subpopulation molecule. Accordingly, natural ligands, derivatives of natural ligands and ***antibodies*** which bind to this 103 gene product, can be utilized to reduce the number of TH2 cells present by either. . . for example, soluble fusion proteins such as Ig-tailed fusion proteins, can be particularly useful for this purpose. In certain instances, ***antibodies*** directed against the 103 gene product, such as directed against the extracellular domain of the 103 gene product, can be. . .

SUMM . . . cell subpopulation-restricted, but the Ig superfamily 200 gene product is, additionally, membrane-bound. Therefore, natural ligands, derivatives of natural ligands and ***antibodies*** which bind to the 200 gene product can be utilized to reduce the number of TH1 cells present by either. . .

SUMM [0055] Further, the Example presented in Section 12, below, describes successful use of ***antibodies*** directed against the 103 gene product as well as 103/Ig fusion proteins to ameliorate symptoms of asthma in an accepted. . .

SUMM . . . gene products described herein are ones also involved in processes related to tissue repair and remodeling after injury, particularly after ***ischemic*** injury. In particular, the example presented in Section 13, below, demonstrates the successful use of ***antibodies*** which bind to the extracellular domain of the 200 gene product to inhibit repair of ***ischemic*** kidney injury. Thus, the invention also makes possible the treatment of ***ischemic*** disorders and injuries. The invention is also based, in part, on the discovery that the 103 gene is expressed in. . .

SUMM [0066] " ***Ischemic*** disorder or injury", as used herein, refers to any disorder or injury to tissues or organs which results from local.

. . .

SUMM [0067] The term "mast-cell related processes or disorders", as used herein, includes, but is not limited to, atherosclerosis and myocardial ***ischemia*** /reperfusion.

DRWD . . . D10.G4, DAX; TH1: AE7.A, Dorris, D1.1). Clones were either unstimulated (-) or stimulated (+) for 6 hours with plate-bound anti-CD3 ***antibody***. Ten micrograms of total RNA were loaded per lane. The positions of 18s and 28s ribosomal RNA are shown as. . .

DRWD . . . 6, brain; lane 7, heart; lane 8, lung; lane 9, spleen; lane 10, liver. Clones were stimulated with plate-bound anti-CD3 ***antibody*** for 6 hours. 7.5 and 10 micrograms total RNA was used for each cell line and each tissue, respectively. a,. . .

DRWD . . . D10.G4, DAX; TH1: AE7.A, Dorris, D1.1). Clones were either unstimulated (-) or stimulated (+) for 6 hours with plate-bound anti-CD3 ***antibody***. The positions of RNA markers, in kilobases, are shown for reference. The arrow marks the position of 200 gene mRNA.

DRWD . . . (D1.1, Dorris, AE7) cell lines and TH2 (D10.G4, DAX, CDC25) cell lines, either stimulated (+) or unstimulated (-) with anti-CD3 ***antibodies***. 15 micrograms of total RNA were loaded per lane. Cells were stimulated between 6 and 7 hours with anti-CD3

antibodies, as described, below, in Section 8.1. The Northern blots were hybridized with a probe made from the entire band 54. . .

DRWD . . . (DAX, CDC25) was isolated from either unstimulated cells or from cells which had been stimulated for two hours with anti-CD3

antibodies. 15 micrograms total RNA were loaded per lane. A band 54 DNA probe was used for hybridization.

DRWD . . . surface 103 gene product. The further to the right the peaks are shifted, the greater the staining intensity, and therefore ***antibody*** binding, exhibited by a cell population.

DRWD . . . 103/Ig fusion results in significant decrease in hallmark symptoms of asthma. FIG. 29A: Animals were treated with the anti-103 3E10 ***antibody*** (listed in the figure as "3E10 MAB"). As a negative control, a set of animals was treated with a non-specific rat Ig ***antibody*** preparation. FIG. 29B: Animals were treated with 103/Ig fusion protein (listed in the figure as "Ig Fus. Prot.") as a negative control, a control set of animals were treated with a non-specific human IgG ***antibody*** preparation.

DRWD . . . a section of untreated mouse kidney tissue; FIG. 31B shows a section of mouse kidney tissue treated with 200 gene ***antibody*** 24 hours prior to, and at 24 hour intervals after the induction of ***ischemic*** kidney injury.

DRWD [0113] FIG. 32. Histological scoring of gene 200 blockage in treated (+a200) and untreated (+RtIg) mouse kidney tissue during renal ***ischemia*** /reperfusion injury (RI), and in non ***ischemic*** controls (S).

DETD . . . rejection and graft versus host disease, are described. The methods and compositions described herein can also be used to treat ***ischemic*** disorders and injuries, including but not limited to, ***ischemic*** renal disease and injury, myocardial ***ischemia*** such as angina pectoris, as well as ***ischemic*** injury to other tissues, including the brain (as in a stroke), spleen, intestine, lung, and testes. Further, the methods and compositions described herein can also be used to regulate ***ischemic*** injury to other types of tissue, such as tumor tissue including, but not limited to tumors of the ovary and. . . gene products of the invention are also involved in processes related to tissue repair and remodeling after injury,

particularly after ***ischemic*** injury. Thus, the genes and gene products of the invention can also be used to successfully treat such injuries and. . .

DETD [0116] Further, the gene products of such fingerprint, target, and pathway genes are described in Section 5.5, ***antibodies*** to such gene products are described in Section 5.6, as are cell- and animal-based models of TH cell subpopulation differentiation. . .

DETD . . . (b) the differentiation and effector function of TH cell subpopulations, and (c) processes related to tissue repair and remodeling after ***ischemic*** injury are described in Section 5.8. Methods for the treatment of immune disorders and ***ischemic*** disorders and injuries are described in Section 5.10.

DETD . . . limited to, pharmacological methods, such as exposure to phorbol esters, calcium ionophores, or lectins (e.g., Concanavalin A), by treatment with ***antibodies*** directed against T-cell receptor epitopes (e.g., anti-CD3 ***antibodies***) or exposure, in the presence of an appropriate antigen presenting cell (APC), to an antigen that the particular TH cells. . .

DETD . . . maintenance, and/or effector function of the subpopulations, using this protein in a manner similar to the well known technique of ***antibody*** probing of .lambda.gt11 libraries.

DETD . . . the pathway and/or differentially expressed genes of the invention also include gene products which are recognized by and bind to ***antibodies*** (polyclonal or monoclonal) directed against the differentially expressed and/or pathway gene products described above; e.g., which are encoded by the. . .

DETD . . . mutant allele. In this manner, gene products made by the putatively mutant tissue can be expressed and screened using standard ***antibody*** screening techniques in conjunction with ***antibodies*** raised against the normal gene product, as described, below, in Section 5.6. (For screening techniques, see, for example, Harlow, E. and Lane, eds., 1988, " ***Antibodies*** : A Laboratory Manual", Cold Spring Harbor Press, Cold Spring Harbor.) In cases where the mutation results in an expressed gene product with altered function (e.g., as a result of a missense mutation), a polyclonal set of ***antibodies*** are likely to cross-react with the mutant gene product. Library clones detected via their reaction with such labeled ***antibodies*** can be purified and subjected to sequence analysis as described in this Section, above.

DETD . . . as many contiguous amino acid residues as necessary to represent an epitope fragment (that is to be recognized by an ***antibody*** directed to the differentially expressed or pathway gene product). For example, such protein fragments or peptides can comprise at least. . .

DETD . . . being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of ***antibodies*** or to screen peptide libraries, for example, vectors which direct the expression of high levels of fusion protein products that. . .

DETD [0229] Alternatively, any fusion protein may be readily purified by utilizing an ***antibody*** specific for the fusion protein being expressed. For example, a system described by Janknecht et al. allows for the ready. . .

DETD [0231] Indirect labeling involves the use of a protein, such as a labeled ***antibody*** , which specifically binds to either a differentially expressed or pathway gene product. Such

antibodies include but are not limited to polyclonal, monoclonal, chimeric, single chain, Fab fragments and fragments produced by an Fab expression. . .

DETD 5.6. ***Antibodies*** Specific for Differentially Expressed or Pathway Gene Products

DETD [0241] Described herein are methods for the production of ***antibodies*** capable of specifically recognizing one or more differentially expressed or pathway gene product epitopes. Such ***antibodies*** can include, but are not limited to, polyclonal ***antibodies***, monoclonal ***antibodies*** (mAbs), humanized or chimeric ***antibodies***, single chain ***antibodies***, Fab fragments, F(ab').sub.2 fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) ***antibodies***, and epitope-binding fragments of any of the above. The Ig tails of such ***antibodies*** can be modified to reduce complement activation and Fc binding. (See, for example, European Patent No. 239400 B1, Aug. 3, .

DETD [0242] Such ***antibodies*** can be used, for example, in the detection of a fingerprint, target, or pathway gene product. in a biological sample, and can be used as part of diagnostic techniques. Alternatively, such ***antibodies*** can be utilized as part of an immune disorder treatment method, as described, below, in Section 5.9. For example, the ***antibodies*** can be used to modulate target gene activity, can be used to modulate TH cell subpopulation differentiation, maintenance and/or effector function, or, in the case of ***antibodies*** directed to cell surface epitopes, can be used to isolate a TH cell subpopulation of interest, for either depletion or.

DETD [0243] Such ***antibodies*** can also be utilized as part of a method for treatment of an ***ischemic*** disorder or injury, as described in Section 5.10.3.1, below. For example, the ***antibodies*** can be used to block or inhibit activity of one or more of the gene products of the invention, thereby reducing or inhibiting repair of certain ***ischemic*** tissues, for example carcinogenic tumors.

DETD [0244] For the production of ***antibodies*** to a differentially expressed or pathway gene, various host animals can be immunized by injection with a differentially expressed or. . .

DETD [0245] Polyclonal ***antibodies*** are heterogeneous populations of ***antibody*** molecules derived from the sera of animals immunized with an antigen, such as target gene product, or an antigenic functional derivative thereof. For the production of polyclonal ***antibodies***, host animals such as those described above, can be immunized by injection with differentially expressed or pathway gene product supplemented. . .

DETD [0246] Monoclonal ***antibodies***, which are homogeneous populations of ***antibodies*** to a particular antigen, can be obtained by any technique which provides for the production of ***antibody*** molecules by continuous cell lines in culture. These include, but are not limited to the hybridoma technique of Kohler and. . . 4:72; Cole et al., 1983, Proc. Natl. Acad. Sci. USA 80:2026-2030), and the EBV-hybridoma technique (Cole et al., 1985, Monoclonal ***Antibodies*** And Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). Such ***antibodies*** can be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD and any subclass thereof. The hybridoma

producing the. . .

DETD [0247] In addition, techniques developed for the production of "chimeric ***antibodies***" (Morrison et al., 1984, Proc. Natl. Acad. Sci., 81:6851-6855; Neuberger et al., 1984, Nature, 312:604-608; Takeda et al., 1985, Nature, 314:452-454; U.S. Pat. No. 4,816,567) by splicing the genes from a mouse ***antibody*** molecule of appropriate antigen specificity together with genes from a human ***antibody*** molecule of appropriate biological activity can be used. A chimeric ***antibody*** is a molecule in which different portions are derived from different animal species, such as those having a variable region.

DETD [0248] Alternatively, techniques described for the production of single chain ***antibodies*** (U.S. Pat. No. 4,946,778; Bird, 1988, Science 242:423-426; Huston et al., 1988, Proc. Natl. Acad. Sci. USA 85:5879-5883; and Ward et al., 1989, Nature 334:544-546) and for making humanized monoclonal ***antibodies*** (U.S. Pat. No. 5,225,539, which is incorporated herein by reference in its entirety) can be utilized to produce anti-differentially expressed or anti-pathway gene product ***antibodies***.

DETD [0249] ***Antibody*** fragments which recognize specific epitopes can be generated by known techniques. For example, such fragments include but are not limited to: the F(ab').sub.2 fragments which can be produced by pepsin digestion of the ***antibody*** molecule and the Fab fragments which can be generated by reducing the disulfide bridges of the F(ab').sub.2 fragments. Alternatively, Fab. . .

DETD [0250] ***Antibodies*** to the differentially expressed or pathway gene products can, in turn, be utilized to generate anti-idiotypic ***antibodies*** that "mimic" such gene products, using techniques well known to those skilled in the art. (See, e.g., Greenspan & Bona, . . Nissinoff, 1991, J. Immunol. 147(8):2429-2438). For example, in the case of receptor-type molecules (e.g., 10, 103 and 200 gene products) ***antibodies*** which bind to the ECD and competitively inhibit the binding of ligand to the receptor can be used to generate.

DETD [0251] Production of ***antibodies*** directed against the extracellular domain of the 103 gene product are described in Section 12, below. Also, production of ***antibodies*** directed against the extracellular domain of the 200 gene product are described in Section 13, below.

DETD . . . from the animal, in situ hybridization analysis, and RT-PCR. Samples of target gene-expressing tissue, can also be evaluated immunocytochemically using ***antibodies*** specific for the target gene transgene gene product of interest.

DETD [0265] The target gene transgenic animals that express target gene mRNA or target gene transgene peptide (detected immunocytochemically, using ***antibodies*** directed against target gene product epitopes) at easily detectable levels can then be further evaluated to identify those animals which. . .

DETD . . . limited to members of random or partially degenerate, directed phosphopeptide libraries; see, e.g., Songyang, Z. et al., 1993, Cell 72:767-778), ***antibodies*** (including, but not limited to polyclonal, monoclonal, humanized, anti-idiotypic, chimeric or single chain ***antibodies***, and FAb, F(ab').sub.2 and FAb expression library fragments, and epitope-binding fragments thereof), and small organic or inorganic molecules. In the. . . bind to the ECD and

either mimic the activity triggered by the natural ligand (i.e., agonists); as well as peptides, ***antibodies*** or fragments thereof, and other organic compounds that mimic the ECD (or a portion thereof) and bind to a "neutralize". . .

DETD . . . can be accomplished by simply coating the solid surface with a solution of the protein and drying. Alternatively, an immobilized ***antibody***, preferably a monoclonal ***antibody***, specific for the protein to be immobilized can be used to anchor the protein to the solid surface. The surfaces. . .

DETD . . . is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; e.g., using a labeled ***antibody*** specific for the previously nonimmobilized component (the ***antibody***, in turn, can be directly labeled or indirectly labeled with a labeled anti-Ig ***antibody***).

DETD . . . be conducted in a liquid phase, the reaction products separated from unreacted components, and complexes detected; e.g., using an immobilized ***antibody*** specific for target gene product or the test compound to anchor any complexes formed in solution, and a labeled ***antibody*** specific for the other component of the possible complex to detect anchored complexes.

DETD . . . target gene protein, especially mutant target gene proteins. Such compounds can include, but are not limited to molecules such as ***antibodies***, peptides, and the like, as described, for example, in Section 5.8.1. above.

DETD . . . coating the solid surface with a solution of the target gene product or binding partner and drying. Alternatively, an immobilized ***antibody*** specific for the species to be anchored can be used to anchor the species to the solid surface. The surfaces. . .

DETD . . . is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; e.g., using a labeled ***antibody*** specific for the initially non-immobilized species (the ***antibody***, in turn, can be directly labeled or indirectly labeled with a labeled anti-Ig ***antibody***). Depending upon the order of addition of reaction components, test compounds which inhibit complex formation or which disrupt preformed complexes. . .

DETD . . . or absence of the test compound, the reaction products separated from unreacted components, and complexes detected; e.g., using an immobilized ***antibody*** specific for one of the binding components to anchor any complexes formed in solution, and a labeled ***antibody*** specific for the other partner to detect anchored complexes. Again, depending upon the order of addition of reactants to the. . .

DETD . . . the resulting fusion protein. The interactive cellular or extracellular binding partner can be purified and used to raise a monoclonal ***antibody***, using methods routinely practiced in the art and described above, in Section 5.6. This ***antibody*** can be labeled with the radioactive isotope ¹²⁵I, for example, by methods routinely practiced in the art. In a heterogeneous. . . binding to occur. At the end of the reaction period, unbound material can be washed away, and the labeled monoclonal ***antibody*** can be added to the system and allowed to bind to the complexed components. The interaction between the target gene. . .

DETD . . . away. Again the extent of inhibition of the target gene product/binding partner interaction can be detected by adding the labeled ***antibody*** and measuring the radioactivity associated

with the beads.

DETD . . . describes the successful utilization of a 103 gene product/Ig fusion protein, as well as the successful use of a monoclonal ***antibody*** directed against the extracellular domain of the 103 gene product to ameliorate symptoms of asthma in an accepted animal model. . .

DETD . . . limited to members of random or partially degenerate, directed phosphopeptide libraries; see, e.g., Songyang, Z. et al., 1993, Cell 72:767-778), ***antibodies*** (including, but not limited to polyclonal, monoclonal, humanized, anti-idiotypic, chimeric or single chain ***antibodies***, and FAb, F(ab').sub.2 and FAb expression library fragments, and epitope-binding fragments thereof), and small organic or inorganic molecules.

DETD . . . soluble Ig-tailed fusion proteins which may be produced by utilizing techniques such as those described, above, in Section 5.5. Additionally, ***antibodies*** directed against the extracellular portion of the 10 gene product may reduce 10 gene product function by, for example, blocking. . .

DETD . . . herein can also be used for treating, ameliorating, or modulating symptoms associated with mast cell-related processes or disorders or certain ***ischemia*** disorders or injuries. For example, techniques which increase the expression or activity of certain gene products of the invention whose activity is involved in the repair of ***ischemic*** injury or damage, particularly techniques which increase the expression or activity of the 200 gene product of this invention, can be used to treat tissue or organ damage produced by an ***ischemic*** disorder or injury. Alternatively, techniques or methods which inhibit the expression or activity of target gene products of the invention can be used to block or inhibit the repair of ***ischemic*** tissue or organs. Such techniques are useful, for example, to treat ***ischemic*** or infarcted tissue, such as a cancerous tumor, to increase damage or injury to such tissue, e.g., during treatment such. . .

DETD [0341] Among the ***ischemic*** disorders or injuries whose symptoms can be ameliorated are ***ischemic*** renal disease and myocardial ***ischemia***, such as angina pectoris, as well as ***ischemic*** injuries to other tissues including, but by no means limited to, the brain (as in a stroke), spleen, intestine, lung, and testes. Such techniques can also be used to treat, or to enhance ***ischemic*** injuries to tumors, including tumors of the ovary or uterus.

DETD [0342] The methods described herein can additionally be used to treat or prevent ***ischemic*** damage or injury to transplanted organs, such as transplanted kidneys, lungs, hearts, livers, and pancreas, or grafts, such as skin grafts. Such techniques are important in that some ***ischemic*** damage to transplanted organs typically occurs during transplant from donor to host, when oxygen perfusion to tissue of the transplanted. . .

DETD . . . is demonstrated in Section 13, herein, to play a critical role in the resolution (i.e., the repair) of injury following ***ischemia*** reperfusion. The 200 gene and its products can, therefore, be utilized in the treatment of ***ischemic*** disorders and injuries. For example, a gene 200 product, or functional portions thereof, can be utilized either directly or indirectly to stimulate or increase the repair of injury to tissue or organs resulting from an ***ischemic*** injury or disorder.

DETD . . . of the present invention, including the 200 gene product, can be used to inhibit the repair or recovery of certain ***ischemic*** tissue.

DETD . . . with the invention to ameliorate certain TH cell subpopulation-related disorder symptoms, or, alternatively, to inhibit the repair or recovery of ***ischemic*** tissue. As discussed in Section 5.8, above, such molecules can include, but are not limited to peptides (such as, for example, peptides representing soluble extracellular portions of target gene product transmembrane receptors), phosphopeptides, small organic or inorganic molecules, or ***antibodies*** (including, for example, polyclonal, monoclonal, humanized, anti-idiotypic, chimeric or single chain ***antibodies***, and FAb, F(ab').sub.2 and FAb expression library fragments, and epitope-binding fragments thereof). In one embodiment, for example, ***antibodies*** directed against a 103 gene product, preferably an extracellular or extracellular portion of a 103 gene product, can be utilized.. . .

DETD . . . IgE immune responses, including, but not limited to the symptoms which accompany atopic conditions such as allergy and/or asthma. IgE-type ***antibodies*** are produced by stimulated B cells which require, at least in part, IL-4 produced by the TH2 cell subpopulation. Therefore,. . .

DETD [0351] For example, natural ligands, derivatives of natural ligands and ***antibodies*** which bind to the 103 gene product can be utilized to reduce the number of TH2 cells present by either. . .

DETD [0357] For example, natural ligands, derivatives of natural ligands and ***antibodies*** which bind to the 200 gene product can be utilized to reduce the number of TH1 cells present by either. . .

DETD . . . Further, reduction in the amount of ligand bound 200 gene transmembrane protein will also inhibit the resolution and repair of ***ischemic*** tissue.

DETD . . . and/or analogs thereof, of the gene 200 product, including, for example, soluble fusion proteins such as Ig-tailed fusion proteins or ***antibodies***. (For a discussion of the production of Ig-tailed fusion proteins see, for example, U.S. Pat. No. 5,116,964.)

DETD . . . these 200 gene product domains or mutants fused to another polypeptide (e.g., an IgFc polypeptide) can be utilized. Alternatively, anti-idiotypic ***antibodies*** or Fab fragments of antiidiotypic ***antibodies*** that mimic the 200 gene product ECD and neutralize 200 gene product ligand can be used. Such 200 gene product peptides, proteins, fusion proteins, anti-idiotypic ***antibodies*** or Fabs are administered to a subject in amounts sufficient to neutralize the gene product and thereby effectuate an amelioration of a T cell subpopulation-related disorder, or an inhibition of repair of ***ischemic*** tissues.

DETD . . . into the tissue site, or modified antisense molecules, designed to target the desired cells (e.g., antisense linked to peptides or ***antibodies*** that specifically bind receptors or antigens expressed on the target cell surface) can be administered systemically.

DETD . . . gene expression or to increase the activity of target gene product can serve to enhance the repair or resolution of ***ischemia*** reperfusion injury to an organ or tissue. Thus, such techniques can also be used to successfully treat ***ischemic*** disorders or injuries.

DETD . . . activity can be used in accordance with the invention to

ameliorate certain TH cell subpopulation-related disorder symptoms, or to treat ***ischemic*** disorders and injuries. As discussed in Section 5.8, above, such molecules can include, but are not limited to proteins or. . . also include peptides representing soluble extracellular portions of target gene product transmembrane proteins, phosphopeptides, small organic or inorganic molecules, or ***antibodies*** (including, for example, polyclonal, monoclonal, humanized, anti-idiotypic, chimeric or single chain ***antibodies***, and FAb, F(ab.sup.1).sub.2 and FAb expression library fragments, and epitope-binding fragments thereof).

DETD . . . symptoms, be administered to a patient exhibiting such symptoms. Such compounds can also be administered to a patient having an ***ischemic*** disorder or injury at a level sufficient to ameliorate the symptoms of the ***ischemic*** disorder or injury. Any of the techniques discussed, below, in Section 5.11, can be utilized for such administration. One of. . .

DETD . . . may therefore be used, e.g., to stimulate or increase the repair of injury to tissue or organs resulting from an ***ischemic*** injury or disorder.

DETD . . . results for the elicitation of a reduction in the immune disorder symptoms, or in a reduction of the symptoms of ***ischemic*** disorders and injuries. In the case of compounds which act intracellularly, the DNA molecules encoding such peptides must be taken.

DETD [0395] In cases wherein such molecules are administered to treat an ***ischemia*** related disorder or injury, preferred techniques are those which serve to selectively administer DNA molecules to the affected organ or tissue. For example, in the case of ***ischemic*** renal disease, techniques for the selective administration of the molecules to cells within the kidney are preferred.

DETD . . . a sufficient circulating concentration of the extracellular molecule for the amelioration of immune disorders, or to ameliorate the symptoms of ***ischemic*** disorders or injuries. Further, as above, when the gene encodes a cell which acts intracellularly or as a transmembrane molecule,. . .

DETD . . . which are sufficient to ameliorate the symptoms of T cell subpopulation related disorders, or, alternatively, to ameliorate the symptoms of ***ischemic*** disorders or injuries. Such cells may be either recombinant or non-recombinant. Among the cells which can be administered to increase. . .

DETD . . . including TH cell subpopulation-related disorders. Further, the modulatory techniques described in Section 5.10.3.1, below, can also be used to treat ***ischemic*** disorders and injuries, or, alternatively, to block or inhibit repair of ***ischemic*** injuries depending on whether such modulation is positive or negative. Thus, in appropriate instances, the procedures of this Section can. . .

DETD 5.10.3.1. ***Antibody*** Techniques

DETD [0402] ***Antibodies*** exhibiting modulatory capability can be utilized to ameliorate immune disorders such as TH cell subpopulation-related disorders, or to treat ***ischemic*** disorders and injuries. Depending on the specific ***antibody***, the modulatory effect can be negative and can, therefore, be utilized as part of the techniques described, above, in Section. . .

DETD [0403] An ***antibody*** having negative modulatory capability refers to an ***antibody*** which specifically binds to and

interferes with the action of a protein. In the case of an extracellular receptor, for example, such an ***antibody*** would specifically bind the extracellular domain of the receptor in a manner which does not activate the receptor but which disrupts the ability of the receptor to bind its natural ligand. For example, ***antibodies*** directed against the extracellular domains of genes 103 or 200 can function as such negative modulators. Additionally, ***antibodies*** directed against one or more of the 10 gene product extracellular domains can function in a negative modulatory manner. Such ***antibodies*** can be generated using standard techniques described in Section 5.6, above, against full length wild type or mutant proteins, or against peptides corresponding to portions of the proteins. The ***antibodies*** include but are not limited to polyclonal, monoclonal, FAb fragments, single chain ***antibodies***, chimeric ***antibodies***, and the like.

DETD [0404] An ***antibody*** having positive modulatory capability refers to an ***antibody*** which specifically binds to a protein and, by binding, serves to, either directly or indirectly, activate the function of the protein which it recognizes. For example, an ***antibody*** can bind to the extracellular portion of a transmembrane protein in a manner which causes the transmembrane protein to function as though its endogenous ligand was binding, thus activating, for example, a signal transduction pathway. ***antibodies*** can be generated using standard techniques described in Section 5.6, above, against full length wild type or mutant proteins, or against peptides corresponding to portions of the proteins. The ***antibodies*** include but are not limited to polyclonal, monoclonal, FAb fragments, single chain ***antibodies***, chimeric ***antibodies***, and the like.

DETD [0405] In instances where the protein, such as a target gene protein, to which the ***antibody*** is directed is intracellular and whole ***antibodies*** are used, internalizing ***antibodies*** can be preferred. However, lipofectin or liposomes can be used to deliver the ***antibody*** or a fragment of the Fab region which binds to the gene product epitope into cells. Where fragments of the ***antibody*** are used, the smallest inhibitory fragment which binds to the protein's binding domain is preferred. For example, peptides having an amino acid sequence corresponding to the domain of the variable region of the ***antibody*** that binds to the protein can be used. Such peptides can be synthesized chemically or produced via recombinant DNA technology. . . . methods well known in the art (e.g., see Creighton, 1983, supra; and Sambrook et al., 1989, above). Alternatively, single chain ***antibodies***, such as neutralizing ***antibodies***, which bind to intracellular epitopes can also be administered. Such single chain ***antibodies*** can be administered, for example, by expressing nucleotide sequences encoding single-chain ***antibodies*** within the target cell population by utilizing, for example, techniques such as those described in Marasco et al. (Marasco, W. . . .

DETD [0406] In instances where the protein to which the ***antibody*** is directed is extracellular, or is a transmembrane protein, any of the administration techniques described, below in Section 5.11 which are appropriate for peptide administration can be utilized to effectively administer the ***antibodies*** to their site of action.

DETD . . . of the separation is to increase or augment the number of cells belonging to a specific TH cell subpopulation, the ***antibodies***

used can also be specific to surface markers present on undifferentiated or partially undifferentiated TH cells. After separation, and purification. . .

DETD . . . such as TH1 or TH2 cells, can be removed, and thus selectively separated and purified, by various methods which utilize ***antibodies*** which bind specific markers present on the T cell population of interest, while absent on other cells within the starting. . . a fluorescence activated cell sorter (FACS) and specific fluorochromes, biotin-avidin or biotin-streptavidin separations using biotin conjugated to cell surface marker-specific ***antibodies*** and avidin or streptavidin bound to a solid support such as affinity column matrix or plastic surfaces or magnetic separations using ***antibody*** -coated magnetic beads.

DETD [0413] Separation via ***antibodies*** for specific markers can be by negative or positive selection procedures. In negative separation, ***antibodies*** are used which are specific for markers present on undesired cells. For example, in the case of a TH1 cell subpopulation-related disorder wherein it would be desirable to deplete the number of TH1 cells, such ***antibodies*** could be directed to the extracellular domain of the 200 gene product. Alternatively, in the case of TH2 cell subpopulation-related disorders wherein it would be desirable to deplete the number of TH1 cells, such ***antibodies*** could be directed to the extracellular domain of the 103 gene product. Cells bound by an ***antibody*** to such a cell surface marker can be removed or lysed and the remaining desired mixture retained.

DETD [0414] In positive separation, ***antibodies*** specific for markers present on the desired cells of interest. For example, in the case of a TH1 cell subpopulation-related disorder wherein it would be desirable to increase the number of TH1 cells, such ***antibodies*** could be directed to the extracellular domain of the 200 gene product. Alternatively, in the case of TH2 cell subpopulation-related disorders wherein it would be desirable to increase the number of TH1 cells, such ***antibodies*** could be directed to the extracellular domain of the 103 gene product. Cells bound by the ***antibody*** are separated and retained. It will be understood that positive and negative separations can be used substantially simultaneously or in. . .

DETD [0415] A common technique for ***antibody*** based separation is the use of flow cytometry such as by a fluorescence activated cell sorter (FACS). Typically, separation by flow cytometry is performed as follows. The suspended mixture of cells are centrifuged and resuspended in media. ***Antibodies*** which are conjugated to fluorochrome are added to allow the binding of the ***antibodies*** to specific cell surface markers. The cell mixture is then washed by one or more centrifugation and resuspension steps. The. . .

DETD . . . such method is biotin-avidin based separation by affinity chromatography. Typically, such a technique is performed by incubating cells with biotin-coupled ***antibodies*** to specific markers, such as, for example, the transmembrane protein encoded by the 103 gene described herein, followed by passage through an avidin column. Biotin-***antibody*** -cell complexes bind to the column via the biotin-avidin interaction, while other cells pass through the column. The specificity of the. . .

DETD . . . proliferation of the purified TH cell subpopulation. Such an expansion agent can, for example, be any appropriate cytokine, antigen, or ***antibody***. In the case of TH2 cells, for example, the

expansion agent can be IL-4, while for TH1 cells, the expansion. . .

DETD [0425] Targeting moieties can include, but are not limited to, ***antibodies*** directed to cell surface markers found specifically on the TH cell subpopulation being targeted, or, alternatively, to ligands, such as. . .

DETD [0426] In the case of TH2 cells, for example, such a targeting moiety can represent an ***antibody*** directed against the extracellular portion of the 103 gene product described herein, or can, alternatively, represent a ligand specific for. . . this receptor-type TH2 specific molecule. In the case of TH1 cells, for example, such a targeting moiety can represent an ***antibody*** directed against the extracellular portion of the 200 gene product described herein, or can, alternatively, represent a ligand specific for. . .

DETD [0431] As defined herein, a therapeutically effective amount of ***antibody***, protein, or polypeptide (i.e., an effective dose or effective dosage) ranges from about 0.001 to 30 mg/kg of body weight,. . .

DETD . . . subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a protein, polypeptide or ***antibody*** can include a single treatment or, preferably, can include a series of treatments. In a preferred example, a subject is treated with ***antibody***, protein, or polypeptide in the range of between about 0.1 to 20 mg/kg body weight one time per week for. . . even more preferably for about 4, 5, or 6 weeks. It will also be appreciated that the effective dosage of ***antibody***, protein or polypeptide used for treatment may increase or decrease over the course of a particular treatment. Changes in dosage. . .

DETD 5.11.3. Pharmaceutical Preparation and Administration of ***Antibodies***

DETD [0444] ***Antibodies*** which specifically bind to target gene products of the invention and thereby modulate their activity can also be administered to a patient at therapeutically effective doses to treat or ameliorate immune disorders, or to treat or ameliorate ***ischemic*** disorders or injuries. For example, Section 13, below, demonstrates the use of anti-200 gene product ***antibodies*** to block recovery of kidney tissue from ***ischemia*** reperfusion injury.

DETD [0445] ***Antibodies*** of the invention are administered by any suitable means, including those described in Section 5.12.2, above. In addition, ***antibody*** to a target gene product of the invention is suitably administered by pulse infusion, particularly with declining doses of the ***antibody***. Preferably, the dosing is administered by injections, most preferably by intravenous or subcutaneous injections, depending in part on whether the. . .

DETD [0446] The appropriate dosage of ***antibody*** will depend on the type of disease to be treated, the severity and course of the disease, whether the ***antibody*** is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the ***antibody***, and the discretion of the attending physician. The ***antibody*** may suitably administered to the patient at one time or, more preferably, over a series of treatments.

DETD [0447] As a general proposition, the initial pharmaceutically effective amount of ***antibody*** administered parenterally will be in the range of about 0.1 to 20 mg/kg of patient body weight per day, with. . .

DETD [0453] Such methods can, for example, utilize reagents such as the fingerprint gene nucleotide sequences described in Sections 5.1, and ***antibodies*** directed against differentially expressed and pathway gene peptides, as described, above, in Sections 5.5 (peptides) and 5.6 (***antibodies***). Specifically, such reagents can be used, for example, for: 1) the detection of the presence of target gene expression, target. . .

DETD . . . performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one specific fingerprint gene nucleic acid or anti-fingerprint gene ***antibody*** reagent described herein, which can be conveniently used, e.g., in clinical settings, to diagnose patients exhibiting TH1- or TH2-related abnormalities.

DETD . . . stimulating the cells prior to contacting the cells with the compound. Among the methods for stimulation are stimulation via anti-CD3 ***antibody*** stimulation.

DETD [0478] ***Antibodies*** directed against wild type or mutant fingerprint gene peptides, which are discussed, above, in Section 5.6, can also be used. . .

DETD . . . employed herein can, for example, be such as those described in Harlow and Lane (Harlow, E. and Lane, D., 1988, " ***Antibodies*** : A Laboratory Manual", Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.), which is incorporated herein by reference in its. . .

DETD . . . molecules can involve, for example, immunoassays wherein fingerprint gene peptides are detected by their interaction with an anti-fingerprint gene product-specific ***antibody*** .

DETD [0481] For example, ***antibodies*** , or fragments of ***antibodies*** , such as those described, above, in Section 5.6, useful in the present invention can be used to quantitatively or qualitatively. . . wild type or mutant fingerprint gene peptides. This can be accomplished, for example, by immunofluorescence techniques employing a fluorescently labeled ***antibody*** (see below, this Section,) coupled with light microscopic, flow cytometric, or fluorimetric detection. Such techniques are especially preferred if the.

DETD [0482] The ***antibodies*** (or fragments thereof) useful in the present invention can, additionally, be employed histologically, as in immunofluorescence or immunoelectron microscopy, for. . . peptides. In situ detection can be accomplished by removing a histological specimen from a patient, and applying thereto a labeled ***antibody*** of the present invention. The ***antibody*** (or fragment) is preferably applied by overlaying the labeled ***antibody*** (or fragment) onto a biological sample. Through the use of such a procedure, it is possible to determine not only. . .

DETD . . . extract, freshly harvested cells, or cells which have been incubated in tissue culture, in the presence of a detectably labeled ***antibody*** capable of identifying fingerprint gene peptides, and detecting the bound ***antibody*** by any of a number of techniques well-known in the art.

DETD . . . soluble proteins. The support can then be washed with suitable buffers followed by treatment with the detectably labeled fingerprint gene-specific ***antibody*** . The solid phase support can then be washed with the buffer a second time to remove unbound ***antibody*** . The amount of bound label on solid support can then be detected by conventional means.

DETD [0485] By "solid phase support or carrier" is intended any support capable of binding an antigen or an ***antibody***. Well-known supports or carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, gabbros, and magnetite.. . . have virtually any possible structural configuration so long as the coupled molecule is capable of binding to an antigen or ***antibody***. Thus, the support configuration can be spherical, as in a bead, or cylindrical, as in the inside surface of a . . . strip, etc. Preferred supports include polystyrene beads. Those skilled in the art will know many other suitable carriers for binding ***antibody*** or antigen, or will be able to ascertain the same by use of routine experimentation.

DETD [0486] The binding activity of a given lot of anti-wild type or mutant fingerprint gene product ***antibody*** can be determined according to well known methods. Those skilled in the art will be able to determine operative and. . .

DETD [0487] One of the ways in which the fingerprint gene peptide-specific ***antibody*** can be detectably labeled is by linking the same to an enzyme and use in an enzyme immunoassay (EIA) (Voller,. . . Raton, Fla.; Ishikawa, E. et al., (eds.), 1981, ENZYME IMMUNOASSAY, Kaku Shoin, Tokyo). The enzyme which is bound to the ***antibody*** will react with an appropriate substrate, preferably a chromogenic substrate, in such a manner as to produce a chemical moiety. . . be detected, for example, by spectrophotometric, fluorimetric or by visual means. Enzymes which can be used to detectably label the ***antibody*** include, but are not limited to, malate dehydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, yeast alcohol dehydrogenase, alpha-glycerophosphate, dehydrogenase, triose phosphate isomerase,. .

DETD [0488] Detection can also be accomplished using any of a variety of other immunoassays. For example, by radioactively labeling the ***antibodies*** or ***antibody*** fragments, it is possible to detect fingerprint gene wild type or mutant peptides through the use of a radioimmunoassay (RIA). . .

DETD [0489] It is also possible to label the ***antibody*** with a fluorescent compound. When the fluorescently labeled ***antibody*** is exposed to light of the proper wavelength, its presence can then be detected due to fluorescence. Among the most. . .

DETD [0490] The ***antibody*** can also be detectably labeled using fluorescence emitting metals such as .sup.152Eu, or others of the lanthanide series. These metals can be attached to the ***antibody*** using such metal chelating groups as diethylenetriaminepentacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA).

DETD [0491] The ***antibody*** also can be detectably labeled by coupling it to a chemiluminescent compound. The presence of the chemiluminescent-tagged ***antibody*** is then determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of. . .

DETD [0492] Likewise, a bioluminescent compound can be used to label the ***antibody*** of the present invention. Bioluminescence is a type of chemiluminescence found in biological systems in, which a catalytic protein increases. . .

DETD . . . samples were isolated from either unstimulated cells or from cells which had been stimulated for 6 hours with plate-bound anti-CD3 ***antibody***. The samples were probed with band 103 sequences, as

shown in FIG. 6. While 103 gene RNA is present in. . .

DETD . . . E. et al., John Wiley & Sons, NY, pp 3.12.4-3.12.6).

Specifically, prior to plating, the flasks were coated with anti-CD3-epsilon. ***antibodies*** (hybridoma supernatant from the 145-C11 hybridoma; Parmingen, Inc., San Diego Calif.). For coating, ***antibodies*** were resuspended in PBS at 1-2 .mu.g/ml at a volume sufficient to coat the bottom of the flasks. Coating solution. . .

DETD [0551] After incubation, the ***antibody*** coating solution was removed by aspiration and cells were immediately added. Flasks were placed in a 37.degree. C. incubator for. . .

DETD . . . samples were isolated from either unstimulated cells (-) or cells which had been stimulated for 6 hours with plate-bound anti-CD3 ***antibody*** (+). The samples were probed with 200 gene sequences, and, as shown in FIG. 18, RNA from both stimulated and. . .

DETD . . . blot data depicted in FIG. 19 additionally illustrates 54 gene expression within cell clones either stimulated or unstimulated with anti-CD3 ***antibodies***, and demonstrates that 54 gene expression goes down within stimulated TH1 cells.

DETD . . . Specifically, RNA was isolated from unstimulated AE7 clones as well as from AE7 clones which had been stimulated with anti-CD3 ***antibodies*** for varying lengths of time, as noted in FIG. 20. As illustrated in FIG. 20, 54 gene expression decreased slightly. . .

DETD . . . herein presents in vivo data demonstrating that the 103 gene product regulates TH2 effector cell responses. In particular, a monoclonal ***antibody*** (3E10 mAb) has been generated against the 103 gene product, and its effect in an adoptive transfer model of TH1.

DETD [0646] Rat monoclonal ***antibodies*** (MAbs), including the 3E10 MAb, were generated against the extracellular domain of the mouse 103 gene product. A DNA sequence. . .

DETD . . . culture supernatant (or 1 .mu.g purified 3E10 protein) was applied 1.times.10.sup.6 cells. After rinsing, cells were contacted with goat anti-rat ***antibody*** conjugated with PE (R-phycoerythrin) fluorescent dye. After a final rinse, cell analysis was carried out on a FACS Vantage (Becton. . .

DETD [0682] To further investigate the expression and role of the 103 gene product in TH cells, a monoclonal ***antibody*** (3E10 mAb) directed against the extracellular domain of the 103 gene product was prepared and characterized.

DETD . . . corroborating the results presented in the Example of Section 7, above. In addition, the data demonstrate the feasibility of using ***antibodies*** to separate TH2 subpopulation cells away from other cell types, thereby modulating a TH cell subpopulation by changing the number. . .

DETD The 200 Gene Product Exhibits a Critical Role in the Resolution of Injury Following Kidney ***Ischemia*** and Reperfusion

DETD . . . The Example provided herein presents in vivo data that the 200 gene product is involved in the recovery from kidney ***ischemia*** injury. In particular, a monoclonal ***antibody*** (96.3.8H7 mAb) generated against the extracellular domain of the murine 200 gene product, and its effect in a surgical model of recovery from kidney ***ischemia*** injury was investigated. The anti-200 mAb markedly impaired restoration of renal function, as determined by several indicators.

DETD . . . vivo animal data demonstrating that the 200 gene product

provides a critical function in the resolution of injury following kidney ***ischemia***. Thus, the 200 gene and its gene product can be used to aid in the recovery from ***ischemic*** injuries such as stroke, heart attack, acute renal failure, and organ transplant.

DETD [0703] Rat monoclonal ***antibodies*** (mAbs) were generated against the extracellular domain of the mouse 200 gene product. Recombinant murine 200 gene IgG1 (m200Ig) fusion. . .

DETD . . . For 200 gene product blockage experiments, mice were pretreated 24 hours before surgery with 100 .mu.g/mouse of rat anti-200 monoclonal ***antibody*** (200 mAb). In control experiments, mice were administered equivalent dosages of rat Ig (RtIg) ***antibody***.

DETD [0708] Mice were then given ***antibody*** at 24 hour intervals following surgical recovery (4 hours post anesthetic).

DETD . . . adhesion molecule called KIM-1 (Ichimura et al., 1998, J. Biol. Chem. 273:4135), levels of which are increased in cells after ***ischemic*** /reperfusion injury. The role of the 200 gene product is ***ischemic*** injury was investigated in vivo using a mouse model of acute renal failure. In particular, this animal model was used to investigate whether neutralization of the 200 gene product in vivo affected tissue repair after an ***ischemic*** injury.

DETD [0713] A monoclonal ***antibody*** (96.3.8H7 mAb) directed against the extracellular domain of the 200 gene product was prepared and characterized, and this ***antibody*** was administered to mice 24 hours before, and at 24 hour intervals following ***ischemic*** kidney injury. Control animals were administered equal dosages of rat Ig at the same intervals. A second group of animals underwent sham surgery, described in Section 13.1 above, wherein the animals were not subjected to ***ischemic*** kidney injury.

DETD . . . 3 below. Creatinine and blood urea nitrogen levels returned to basal levels in untreated (+RtIg) mice within 72 hours post ***ischemia***. However, mice treated with anti-200 mAb (+a200) maintained elevated levels of both blood urea nitrogen (122.5 mg/dl vs. 34.3 mg/dl). . .

DETD . . . these results provide in vivo animal data demonstrating that the 200 gene product plays a critical role during recovery from ***ischemic*** kidney injury, and can be used as a novel treatment to aid in the recovery from ***ischemic*** injuries such as acute renal failure.

DETD . . . high levels of 103 gene expression in a human mast cell line. The example also describes the production of monoclonal ***antibodies*** which are specific for the human, but not mouse, 103 gene product. FAC staining of the human mast cell line demonstrated binding of these monoclonal ***antibodies***, confirming that the 103 gene product is, indeed, expressed in mast cells.

DETD [0719] Monoclonal ***antibodies*** (mAbs; see below) were generated against the extracellular domain (amino acid residues 18-323) of a human 103 gene product (with. . .

DETD . . . protein using fluorescence activated cells sorting (FACS) according to standard methods described in Section 12.1, above using anti-mouse IgFITC secondary ***antibodies***.

DETD . . . in a human mast cell line. Expression of the 103 gene product in this cell line was verified using monoclonal ***antibodies*** raised against an Fc fusion protein of the human 103 gene product, as described in Section 14.1, above.

DETD [0732] FACS staining of the human mast cell line, with the 21 monoclonal

antibodies showed staining with 15 of the 21 ***antibodies*** compared to isotype controls. Five of these 15 ***antibodies***, identified as 1B4, 2O3, 3F7, 3H18, and 10F7, were selected for further analysis. FAC staining with these ***antibodies*** was demonstrated to be specifically blocked with an excess of human 103-Fc fusion protein, however, staining was not blocked with. . .

DETD . . . is expressed in a human mast cell line. Accordingly, the 103 gene, its gene product, and compositions derived therefrom (e.g., ***antibodies*** and other compounds which bind to and/or modulate the expression or activity of the 103 gene or its gene product). . . include, but are not limited to, atherosclerosis (see, e.g., Metzler and Xu, 1997, Int. Arch. Allergy Immunol. 114:10-14) and myocardial ***ischemia*** /reperfusion (see, e.g., Frangogiannis et al., 1998, Circulation 98:699-710).

CLM What is claimed is:

1. A method for ameliorating a symptom of an ***ischemic*** disorder or injury in a mammal, comprising administering to the mammal a 200 gene product in an amount effective to ameliorate the symptom of the ***ischemic*** disorder or injury.

2. A method for ameliorating a symptom of an ***ischemic*** disorder or injury in a mammal, comprising administering to the mammal a nucleic acid molecule encoding a 200 gene product in an amount effective to ameliorate the symptom of the ***ischemic*** disorder or injury.

3. A method for ameliorating a symptom of an ***ischemic*** disorder or injury in a mammal, comprising administering to the mammal an ***antibody*** directed against a 200 gene product in an amount effective to ameliorate the symptom of the disorder.

4. The method of claim 1, 2, or 3, wherein the ***ischemic*** disorder is ***ischemic*** renal disease, or myocardial ***ischemia***.

5. The method of claim 4, wherein the myocardial ***ischemia*** is angina pectoris.

6. The method of claim 1, 2, or 3 wherein the ***ischemic*** disorder or injury is a infarction.

8. The method of claim 1, 2, or 3, wherein the ***ischemic*** injury is to a transplanted organ.

17. The method of claim 3 wherein said administering of the ***antibody*** is parenteral, subcutaneous, intraperitoneal, intrapulmonary, intranasal, or intralesional.

18. The method of claim 17, wherein the intralesional administration comprises perfusing or contacting a graft or organ with the ***antibody*** before transplant.

19. The method of claim 3, wherein the amount of the ***antibody*** administered is from about 1 .mu.g/kg to about 100 mg/kg.

20. The method of claim 19, wherein the amount of the ***antibody***

administered is from about 1 .mu.g/kg to about 15 mg/kg.

21. The method of claim 20, wherein the amount of the ***antibody*** administered is from about 0.1 mg/kg to about 2.0 mg/kg.

L9 ANSWER 7 OF 9 USPATFULL on STN
AN 2003:99208 USPATFULL
TI Novel human dickkopf-related protein and nucleic acid molecules and uses therefor
IN ***McCarthy, Sean A.*** , San Diego, CA, UNITED STATES
PA Millennium Pharmaceuticals, Inc. (U.S. corporation)
PI US 2003068312 A1 20030410
AI US 2001-972473 A1 20011004 (9)
RLI Continuation of Ser. No. US 1999-263022, filed on 5 Mar 1999, ABANDONED
PRAI WO 1998-US7894 19980416
US 1998-71589P 19980115 (60)
DT Utility
FS APPLICATION
LREP LAHIVE & COCKFIELD, 28 STATE STREET, BOSTON, MA, 02109
CLMN Number of Claims: 22
ECL Exemplary Claim: 1
DRWN 29 Drawing Page(s)
LN.CNT 7186
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Novel Dkk and Dkk-related polypeptides, proteins, and nucleic acid molecules are disclosed. In addition to isolated, full-length Dkk and Dkk-related proteins, the invention further provides isolated fusion proteins, antigenic peptides and ***antibodies***. The invention also provides Dkk and Dkk-related nucleic acid molecules, recombinant expression vectors containing a nucleic acid molecule of the invention, host cells into which the expression vectors have been introduced and non-human transgenic animals in which a Dkk and Dkk-related gene has been introduced or disrupted. Diagnostic, screening and therapeutic methods utilizing compositions of the invention are also provided.
IN ***McCarthy, Sean A.*** , San Diego, CA, UNITED STATES
AB . . . disclosed. In addition to isolated, full-length Dkk and Dkk-related proteins, the invention further provides isolated fusion proteins, antigenic peptides and ***antibodies***. The invention also provides Dkk and Dkk-related nucleic acid molecules, recombinant expression vectors containing a nucleic acid molecule of the . . .
SUMM . . . thereof, can be operatively linked to a non-Dkk polypeptide or non-Dkk-related polypeptide to form fusion proteins. The invention further features ***antibodies*** that specifically bind Dkk or Dkk-related proteins, such as monoclonal or polyclonal ***antibodies***. In addition, the proteins or biologically active portions thereof can be incorporated into pharmaceutical compositions, which optionally include pharmaceutically acceptable. . .
SUMM . . . Dkk-related activity). In another embodiment, the agent stimulates Dkk activity (or Dkk-related activity). In one embodiment, the agent is an ***antibody*** that specifically binds to a Dkk (or Dkk-related) protein. In another embodiment, the agent modulates expression of a protein (e.g., . . .
SUMM . . . another embodiment the modulator is a Dkk or Dkk-related nucleic acid molecule. In yet another embodiment, the modulator is an

antibody peptide, peptidomimetic, or other small molecule. In a preferred embodiment, the disorder characterized by aberrant protein or nucleic acid expression. . .

DETD . . . receptors or antigens expressed on a selected cell surface, e.g., by linking the antisense nucleic acid molecules to peptides or ***antibodies*** which bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using.

DETD [0113] II. Isolated Dkk Proteins and Anti-Dkk ***Antibodies***

DETD . . . proteins, Dkk-related proteins and biologically active portions thereof, as well as polypeptide fragments suitable for use as immunogens to raise ***antibodies***. In one embodiment, native Dkk or Dkk-related proteins can be isolated from cells or tissue sources by an appropriate purification. . .

DETD . . . adhesion, and/or cell fate. Moreover, the Dkk-immunoglobulin fusion proteins of the invention can be used as immunogens to produce anti-Dkk ***antibodies*** in a subject, to purify Dkk ligands and in screening assays to identify molecules which inhibit the interaction of Dkk. . .

DETD . . . An isolated Dkk protein, Dkk-related protein, or a portion or fragment thereof, can be used as an immunogen to generate ***antibodies*** that bind Dkk or Dkk-related proteins using standard techniques for polyclonal and monoclonal ***antibody*** preparation. A full-length Dkk or Dkk-related protein can be used or, alternatively, the invention provides antigenic peptide fragments for use. . . NO:8, SEQ ID NO:14, or SEQ ID NO:21 and encompasses an epitope of Dkk or Dkk-related protein such that an ***antibody*** raised against the peptide forms a specific immune complex with the protein. Preferably, the antigenic peptide comprises at least 10. . .

DETD [0140] A Dkk or Dkk-related immunogen typically is used to prepare ***antibodies*** by immunizing a suitable subject, (e.g., rabbit, goat, mouse or other mammal) with the immunogen. An appropriate immunogenic preparation can. . . or similar immunostimulatory agent. Immunization of a suitable subject with an immunogenic Dkk preparation, for example, induces a polyclonal anti-Dkk ***antibody*** response.

DETD [0141] Accordingly, another aspect of the invention pertains to anti-Dkk ***antibodies*** as well as antibodies to Dkk-related proteins. The term " ***antibody*** " as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen. . . Examples of immunologically active portions of immunoglobulin molecules include F(ab) and F(ab').sub.2 fragments which can be generated by treating the ***antibody*** with an enzyme such as pepsin. The invention provides polyclonal and monoclonal ***antibodies*** that bind Dkk or Dkk-related polypeptides. The term "monoclonal ***antibody*** " or "monoclonal ***antibody*** composition", as used herein, refers to a population of ***antibody*** molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of Dkk or a or Dkk-related protein. A monoclonal ***antibody*** composition thus typically displays a single binding affinity for a particular Dkk or Dkk-related protein with which it immunoreacts.

DETD [0142] Polyclonal ***antibodies*** can be prepared as described above by immunizing a suitable subject with a Dkk or Dkk-related immunogen. The ***antibody*** titer in the immunized subject can be

monitored over time by standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using immobilized Dkk or Dkk-related protein. If desired, the ***antibody*** molecules directed against Dkk or Dkk-related protein can be isolated from the mammal (e.g., from the blood) and further purified. . . techniques, such as protein A chromatography to obtain the IgG fraction. At an appropriate time after immunization, e.g., when the ***antibody*** titers are highest, ***antibody*** -producing cells can be obtained from the subject and used to prepare monoclonal ***antibodies*** by standard techniques, such as the hybridoma technique originally described by Kohler and Milstein (1975) *Nature* 256:495-497) (see also, Brown. . . human B cell hybridoma technique (Kozbor et al, (1983) *Immunol Today* 4:72), the EBV-hybridoma technique (Cole et al, (1985), *Monoclonal ***Antibodies*** and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96) or trioma techniques. The technology for producing monoclonal ***antibody*** hybridomas is well known (see generally R. H. Kenneth, in *Monoclonal ***Antibodies*** : A New Dimension In Biological Analyses*, Plenum Publishing Corp., New York, N.Y. (1980); E. A. Lerner (1981) *Yale J Biol.* . . . described above, and the culture supernatants of the resulting hybridoma cells are screened to identify a hybridoma producing a monoclonal ***antibody*** that binds Dkk or Dkk-related protein.

DETD . . . used for fusing lymphocytes and immortalized cell lines can be applied for the purpose of generating a monoclonal 1 5 ***antibody*** (see, e.g., G. Galfre et al., (1 977) *Nature* 266:55052; Gefter et al, *Somatic Cell Genet.*, cited supra; Lerner, *Yale J. Biol. Med.*, cited supra; Kenneth, *Monoclonal ***Antibodies****, cited supra). Moreover, the ordinarily skilled worker will appreciate that there are many variations of such methods which also would. . . unproductively fused myeloma cells (unfused splenocytes die after several days because they are not transformed). Hybridoma cells producing a monoclonal ***antibody*** of the invention are detected by screening the hybridoma culture supernatants for ***antibodies*** that bind Dkk or Dkk-related protein, e.g., using a standard ELISA assay.

DETD [0144] Alternative to preparing monoclonal ***antibody*** -secreting hybridomas, a monoclonal ***antibody*** can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (e.g., an ***antibody*** phage display library) with Dkk or Dkk-related protein to thereby isolate immunoglobulin library members that bind Dkk or Dkk-related protein. Kits for generating and screening phage display libraries are commercially available (e.g., the Pharmacia Recombinant Phage ***Antibody*** System, Catalog No. 27-9400-01; and the Stratagene SurfZAP.TM. Phage Display Kit, Catalog No. 240612). Additionally, examples of methods and reagents particularly amenable for use in generating and screening ***antibody*** display library can be found in, for example, Ladner et al., U.S. Pat. No. 5,223,409; Kang et al., PCT International. . . Ladner et al., PCT International Publication No. WO 90/02809; Fuchs et al., (1991) *Bio/Technology* 9:1370-1372; Hay et al., (1992) *Hum. ***Antibod*** . Hybridomas* 3:81-85; Huse et al., (1989) *Science* 246:1275-1281; Griffiths et al., (1993) *EMBO J* 12:725-734; Hawkins et al., (1992) *J. . .*

DETD [0145] Additionally, recombinant ***antibodies***, such as chimeric and humanized monoclonal ***antibodies***, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the invention. Such chimeric and

humanized monoclonal ***antibodies*** can be produced by recombinant DNA techniques known in the art, for example using methods described in Robinson et al., . . .

DETD [0146] An ***antibody*** (e.g., monoclonal ***antibody***) can be used to isolate Dkk or Dkk-related protein by standard techniques, such as affinity chromatography or immunoprecipitation. An antibody can. . . Dkk or Dkk-related protein from cells and of recombinantly produced Dkk or Dkk-related protein expressed in host cells. Moreover, an ***antibody*** can be used to detect Dkk or Dkk-related protein (e.g., in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the Dkk or Dkk-related protein. ***Antibodies*** can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, e.g., to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (i.e., physically linking) the ***antibody*** to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and. . .

DETD . . . be utilized in activity assays, in ligand binding (e.g., direct assays or competitive assays described in detail below), to generate ***antibodies*** specific for Dkk or Dkk-related proteins, as examples. In a preferred embodiment, a Dkk or Dkk-related fusion expressed in a. . .

DETD [0171] The Dkk and Dkk-related nucleic acid molecules, Dkk and Dkk-related proteins, and anti-Dkk or anti-Dkk-related protein ***antibodies*** (also referred to herein as "active compounds") of the invention can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the nucleic acid molecule, protein, or ***antibody*** and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all. . .

DETD [0174] Sterile injectable solutions can be prepared by incorporating the active compound (e.g., a Dkk protein, Dkk-related protein or ***antibody***) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed. . .

DETD . . . be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal ***antibodies*** to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to. . .

DETD [0186] The molecules of the present invention (e.g., nucleic acid molecules, proteins, protein homologues, and ***antibodies*** described herein) can be used in one or more of the following methods: a) screening assays; b) predictive medicine (e.g.,. . .

DETD . . . characterized by abnormal cell differentiation and/or survival, an abnormal extracellular structure, or an abnormality in a defense mechanism). Moreover, the ***antibodies*** of the invention can be used to detect and isolate Dkk or Dkk-related proteins, regulate the bioavailability of Dkk or. . .

DETD . . . (e.g., biotinylation kit, Pierce Chemicals, Rockford, IL), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, ***antibodies*** reactive with Dkk, Dkk-related protein, or target molecules but which do not interfere with

binding of the protein to its. . . be derivatized to the wells of the plate, and unbound target, Dkk, or Dkk-related protein trapped in the wells by ***antibody*** conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using ***antibodies*** reactive with the Dkk or Dkk-related protein or target molecule, as well as enzyme-linked assays which rely on detecting an. . .

DETD . . . example, an agent identified as described herein (e.g., a Dkk modulating agent, an antisense Dkk nucleic acid molecule, a Dkk-specific ***antibody***, or a Dkk-binding partner) can be used in an animal model to determine the efficacy, toxicity, or side effects of. . .

DETD [0242] A preferred agent for detecting Dkk or Dkk-related protein is an ***antibody*** capable of binding to the protein, preferably an ***antibody*** with a detectable label. ***Antibodies*** can be polyclonal, or more preferably, monoclonal. An intact ***antibody***, or a fragment thereof (e.g., Fab or F(ab').sub.2) can be used. The term "labeled", with regard to the probe or ***antibody***, is intended to encompass direct labeling of the probe or ***antibody*** by coupling (i.e., physically linking) a detectable substance to the probe or ***antibody***, as well as indirect labeling of the probe or ***antibody*** by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary ***antibody*** using a fluorescently labeled secondary ***antibody*** and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin. The term. . .
. . . Southern hybridizations. Furthermore, in vivo techniques for detection of Dkk or Dkk-related protein include introducing into a subject a labeled ***antibody***. For example, the ***antibody*** can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging. . .

DETD . . . described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or ***antibody*** reagent described herein, which may be conveniently used, e.g., in clinical settings to diagnose patients exhibiting symptoms or family history. . .

DETD . . . more Dkk or Dkk-related protein activity. Examples of such inhibitory agents include antisense Dkk or Dkk-related nucleic acid molecules and ***antibodies***. These modulatory methods can be performed in vitro (e.g., by culturing the cell with the agent) or, alternatively, in vivo. . .

DETD . . . and skeletal muscle. Accordingly, Dkk-3 polypeptides, nucleic acids, or modulators thereof, can be used to treat cardiovascular disorders, such as ***ischemic*** heart disease (e.g., angina pectoris, myocardial infarction, and chronic ***ischemic*** heart disease), hypertensive heart disease, pulmonary heart disease, valvular heart disease (e.g., rheumatic fever and rheumatic heart disease, endocarditis, mitral. . .

DETD . . . such as diseases associated with amaurosis (e.g., a. fugax and a. albuminuric) diseases associated with amblyopia, glaucoma, optic neuropathy (e.g., ***ischemic*** neuropathy, optic neuritis, and infiltrative neuropathy), ophthalmia (e.g., o. catarrhal, trachoma, o. neuroparalytic, and conjunctiva), visual disorders resulting from systemic. . .

DETD . . . to, e.g., infection, toxins, or drugs), inflammations (e.g., bacterial and viral meningitis, encephalitis, and cerebral

toxoplasmosis), cerebrovascular diseases (e.g., hypoxia, ***ischemia***, and infarction, intracranial hemorrhage and vascular malformations, and hypertensive encephalopathy), and tumors (e.g., neuroglial tumors, neuronal tumors, tumors of pineal. . .

DETD . . . and skeletal muscle. Accordingly, Dkk-2 polypeptides, nucleic acids, or modulators thereof, can be used to treat cardiovascular disorders, such as ***ischemic*** heart disease (e.g., angina pectoris, myocardial infarction, and chronic ***ischemic*** heart disease), hypertensive heart disease, pulmonary heart disease, valvular heart disease (e.g., rheumatic fever and rheumatic heart disease, endocarditis, mitral. . .

DETD . . . to, e.g., infection, toxins, or drugs), inflammations (e.g., bacterial and viral meningitis, encephalitis, and cerebral toxoplasmosis), cerebrovascular diseases (e.g., hypoxia, ***ischemia***, and infarction, intracranial hemorrhage and vascular malformations, and hypertensive encephalopathy), and tumors (e.g., neuroglial tumors, neuronal tumors, tumors of pineal. . .

DETD . . . disorder associated with a Dkk or Dkk-related protein which includes the step of administering a therapeutically effective amount of an ***antibody*** to a Dkk or Dkk-related protein to a subject. As defined herein, a therapeutically effective amount of ***antibody*** (i.e., an effective dosage) ranges from about 0.001 to 30 mg/kg body weight, preferably about 0.01 to 25 mg/kg body. . . age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of an ***antibody*** can include a single treatment or, preferably, can include a series of treatments. In a preferred example, a subject is treated with ***antibody*** in the range of between about 0.1 to 20 mg/kg body weight, one time per week for between about 1. . . even more preferably for about 4, 5, or 6 weeks. It will also be appreciated that the effective dosage of ***antibody*** used for treatment may increase or decrease over the course of a particular treatment. Changes in dosage may result from. . .

DETD . . . to: spinal cord injuries, brain injuries, brain tumors (e.g., astrocytic tumors, for example, astrocytomas and glioblastomas), lesions associated with surgery, ***ischemic*** lesions, malignant lesions, infectious lesions, degenerative lesions (e.g., Parkinson's disease, Alzheimer's disease, Huntington's chorea, amyotrophic lateral sclerosis), demyelinating diseases (e.g.,. . .

DETD . . . gel buffer, run out on a 4-20% SDS-PAGE gel, transferred to a nylon membrane and probed with the anti-FLAG monoclonal ***antibody*** M2. Samples from both supernatant and pellet samples showed significant immunoreactivity within a molecular weight range of 40-65 kDa on autoradiographic film using a HRP conjugated secondary ***antibody*** and ECL detection reagents. Thus, both forms of hDkk-3 tested are secreted from 293T cells thereby confirming experimentally that hDkk-3.

DETD . . . blotting. For western analysis, samples were electroblotted onto PVDF (Novex) after SDS-PAGE on 4-20% gradient gels, probed with M2 anti-flag ***antibody*** (1:500, Sigma) followed by HRP conjugated sheep anti-mouse IgG (1:5000, Amersham), developed with chemiluminescent reagents (Renaissance, Dupont) and exposed to. . .

DETD . . . exactly to the predicted signal peptide cleavage site (between Ala-18 and Leu-19). Because the same band is identified by anti-flag ***antibodies***, which recognize the C-terminal epitope tag, band (i)

was thus identified as the full length, mature hDkk-4 protein. The band.

DET D Preparation of ***Antibodies*** Specific for hDkk and hSoggy Proteins

DET D [0397] This example describes the making of polyclonal ***antibodies*** specific for hDkk-1, hDkk-4, hDkk-1, hDkk-2, and hSoggy-1.

DET D [0400] ***Antibodies*** were generated in New Zealand white rabbits over a 10-week period. The immunogen includes KLH-peptide emulsified by mixing with. . .

DET D . . . purification, peptide antigens were immobilized on an activated support. Antisera was passed through the sera column and then washed. Specific ***antibodies*** were eluted via a pH gradient, collected and stored in a borate buffer (0.125M total borate) at about 0.25mg/ml. The. . .

DET D [0402] All ***antibodies*** performed well in ELISA assays. Anti-peptide #44, #46, and #58 are particularly useful for detection of hDkk-3 and hSoggy-1, respectively,. . .

CLM What is claimed is:

11. An ***antibody*** which selectively binds to a polypeptide of claim 8.

14. The method of claim 13, wherein the compound which binds to the polypeptide is an ***antibody***.

L9 ANSWER 8 OF 9 USPATFULL on STN

AN 2001:205588 USPATFULL

TI Novel SSP-1 compositions and therapeutic and diagnostic uses therefor

IN ***McCarthy, Sean Anthony***, Boston, MA, United States

PA Millennium Biotherapeutics (U.S. corporation)

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RLI Continuation of Ser. No. US 1997-843656, filed on 16 Apr 1997, UNKNOWN

DT Utility

FS APPLICATION

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CLMN Number of Claims: 61

ECL Exemplary Claim: 1

DRWN 3 Drawing Page(s)

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CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to the discovery of novel genes encoding Small Secreted Protein-1 (SSP-1) polypeptides. Therapeutics, diagnostics and screening assays based on these molecules are also disclosed.

IN ***McCarthy, Sean Anthony***, Boston, MA, United States

SUMM . . . preparation, the immunogen being capable of eliciting an immune response specific for a SSP-1 polypeptide; e.g. a humoral response, an ***antibody*** response and/or cellular response. In a preferred embodiment, the immunogen comprises an antigenic determinant, e.g. a unique determinant of a. . .

SUMM [0022] A still further aspect of the present invention features ***antibodies*** and ***antibody*** preparations specifically reactive with an epitope of a SSP-1 protein.

SUMM . . . ligation chain reaction (LCR). In alternate embodiments, the level of a SSP-1 protein is detected in an immunoassay using an ***antibody*** which is specifically immunoreactive with a wild-type or mutated SSP-1 protein.

DRWD . . . nucleic acid molecules encoding SSP-1 proteins, antisense molecules, ribozymes and triplex molecules that block expression of SSP-1 genes, SSP-1 proteins, ***antibodies*** immunoreactive with SSP-1 proteins, and preparations of such immunogenic compositions. In addition, the present invention relates to therapies, which are. . .

DRWD . . . of a SSP-1 gene. Antigenic functions include possession of an epitope or antigenic site that is capable of cross-reacting with ***antibodies*** raised against a naturally occurring or denatured SSP-1 polypeptide or fragment thereof.

DRWD . . . expression in a cell expression a bioactivity of SSP-1. Modulating agents of the present invention can be nucleic acids, polypeptides, ***antibodies***, or compounds.

DRWD . . . into the tissue site, or modified antisense molecules, designed to target the desired cells (e.g., antisense linked to peptides or ***antibodies*** that specifically bind receptors or antigens expressed on the target cell surface) can be administered systematically.

DRWD . . . techniques known in the art for purifying proteins including ion-exchange chromatography, gel filtration chromatography, ultrafiltration, electrophoresis, and immunoaffinity purification with ***antibodies*** specific for such peptide. In a preferred embodiment, the recombinant SSP-1 polypeptide is a fusion protein containing a domain which. . .

DRWD . . . form of a viral particle. The nucleic acid sequences corresponding to the portion of a subject SSP-1 protein to which ***antibodies*** are to be raised can be incorporated into a fusion gene construct which includes coding sequences for a late vaccinia. .

DRWD [0160] ***Antibodies***

DRWD [0161] Another aspect of the invention pertains to an ***antibody*** specifically reactive with a mammalian SSP-1 protein. For example, by using immunogens derived from a SSP-1 protein, e.g., based on the cDNA sequences, anti-protein/anti-peptide antisera or monoclonal ***antibodies*** can be made by standard protocols (See, for example, ***Antibodies*** : A Laboratory Manual ed. by Harlow and Lane (Cold Spring Harbor Press: 1988)). A mammal, such as a mouse, a . . . immunogenic form of the peptide (e.g., a mammalian SSP-1 polypeptide or an antigenic fragment which is capable of eliciting an ***antibody*** response, or a fusion protein as described above). Techniques for conferring immunogenicity on a protein or peptide include conjugation to. . . SSP-1 protein can be administered in the presence of adjuvant. The progress of immunization can be monitored by detection of ***antibody*** titers in plasma or serum. Standard ELISA or other immunoassays can be used with the immunogen as antigen to assess the levels of ***antibodies***. In a preferred embodiment, the subject ***antibodies*** are immunospecific for antigenic determinants of a SSP-1 protein of a mammal, e.g., antigenic determinants of a protein set forth. . .

DRWD . . . animal with an antigenic preparation of a SSP-1 polypeptide, anti- SSP-1 antisera can be obtained and, if desired, polyclonal anti-SSP-1 ***antibodies*** isolated from the serum. To produce

monoclonal ***antibodies***, ***antibody***-producing cells (lymphocytes) can be harvested from an immunized animal and fused by standard somatic cell fusion procedures with immortalizing cells. . . B cell hybridoma technique (Kozbar et al., (1983) Immunology Today, 4: 72), and the EBV-hybridoma technique to produce human monoclonal ***antibodies*** (Cole et al., (1985) Monoclonal ***Antibodies*** and Cancer Therapy, Alan R. Liss, Inc. pp. 77-96). Hybridoma cells can be screened immunochemically for production of ***antibodies*** specifically reactive with a mammalian SSP-1 polypeptide of the present invention and monoclonal ***antibodies*** isolated from a culture comprising such hybridoma cells. In one embodiment anti-human SSP-1 ***antibodies*** specifically react with the protein encoded by the DNA of ATCC deposit No.

DRWD [0163] The term ***antibody*** as used herein is intended to include fragments thereof which are also specifically reactive with one of the subject mammalian SSP-1 polypeptides. ***Antibodies*** can be fragmented using conventional techniques and the fragments screened for utility in the same manner as described above for whole ***antibodies***. For example, F(ab).sub.2 fragments can be generated by treating ***antibody*** with pepsin. The resulting F(ab).sub.2 fragment can be treated to reduce disulfide bridges to produce Fab fragments. The ***antibody*** of the present invention is further intended to include bispecific, single-chain, and chimeric and humanized molecules having affinity for a SSP-1 protein conferred by at least one CDR region of the ***antibody***. In preferred embodiments, the ***antibodies***, the ***antibody*** further comprises a label attached thereto and able to be detected, (e.g., the label can be a radioisotope, fluorescent compound,. . .

DRWD [0164] Anti-SSP-1 ***antibodies*** can be used, e.g., to monitor SSP-1 protein levels in an individual for determining, e.g., whether a subject has a. . .

DRWD [0165] Another application of anti-SSP-1 ***antibodies*** of the present invention is in the immunological screening of cDNA libraries constructed in expression vectors such as .lambda.gt11, .lambda.gt18-23,. . . e.g., other orthologs of a particular SSP-1 protein or other paralogs from the same species, can then be detected with ***antibodies***, as, for example, reacting nitrocellulose filters lifted from infected plates with anti-SSP-1 ***antibodies***. Positive phage detected by this assay can then be isolated from the infected plate. Thus, the presence of SSP-1 homologs. . .

DRWD . . . Disorders of the nervous system include, but are not limited to: spinal cord injuries, brain injuries, lesions associated with surgery, ***ischemic*** lesions, malignant lesions, infectious lesions, degenerative lesions (Parkinson's disease, Alzheimer's disease, Huntington's chorea, amyotrophic lateral sclerosis), demyelating diseases (multiple sclerosis,. . .

DRWD . . . described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or ***antibody*** reagent described herein, which may be conveniently used, e.g., in clinical settings to diagnose patients exhibiting symptoms or family history. . .

DRWD [0205] ***Antibodies*** directed against wild type or mutant SSP-1 proteins, which are discussed, above, may also be used in disease diagnostics and. . . herein may also be such as those described in Harlow and Lane, for example, (Harlow, E. and Lane, D., 1988, "

Antibodies : A Laboratory Manual", Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.), which is incorporated herein by reference in its. . .

DRWD [0206] This can be accomplished, for example, by immunofluorescence techniques employing a fluorescently labeled ***antibody*** (see below) coupled with light microscopic, flow cytometric, or fluorimetric detection. The ***antibodies*** (or fragments thereof) useful in the present invention may, additionally, be employed histologically, as in immunofluorescence or immunoelectron microscopy, for. . . proteins. In situ detection may be accomplished by removing a histological specimen from a patient, and applying thereto a labeled ***antibody*** of the present invention. The ***antibody*** (or fragment) is preferably applied by overlaying the labeled ***antibody*** (or fragment) onto a biological sample. Through the use of such a procedure, it is possible to determine not only. . .

DRWD [0207] Often a solid phase support or carrier is used as a support capable of binding an antigen or an ***antibody***. Well-known supports or carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, gabbros, and magnetite.. . . have virtually any possible structural configuration so long as the coupled molecule is capable of binding to an antigen or ***antibody***. Thus, the support configuration may be spherical, as in a bead, or cylindrical, as in the inside surface of a. . . strip, etc. Preferred supports include polystyrene beads. Those skilled in the art will know many other suitable carriers for binding ***antibody*** or antigen, or will be able to ascertain the same by use of routine experimentation.

DRWD [0208] One means for labeling an anti-SSP-1 protein specific ***antibody*** is via linkage to an enzyme and use in an enzyme immunoassay (EIA) (Voller, "The Enzyme Linked Immunosorbent Assay (ELISA)",. . . Raton, Fla., 1980; Ishikawa, et al., (eds.) Enzyme Immunoassay, Kaku Shoin, Tokyo, 1981). The enzyme which is bound to the ***antibody*** will react with an appropriate substrate, preferably a chromogenic substrate, in such a manner as to produce a chemical moiety. . . be detected, for example, by spectrophotometric, fluorimetric or by visual means. Enzymes which can be used to detectably label the ***antibody*** include, but are not limited to, malate dehydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, yeast alcohol dehydrogenase, alphas-glycerophosphate dehydrogenase, triose phosphate isomerase,. . .

DRWD [0209] It is also possible to label the ***antibody*** with a fluorescent compound. When the fluorescently labeled ***antibody*** is exposed to light of the proper wave length, its presence can then be detected due to fluorescence. Among the. . .

DRWD [0210] The ***antibody*** can also be detectably labeled using fluorescence emitting metals such as ¹⁵²Eu, or others of the lanthanide series. These metals can be attached to the ***antibody*** using such metal chelating groups as diethylenetriaminepentaacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA).

DRWD [0211] The ***antibody*** also can be detectably labeled by coupling it to a chemiluminescent compound. The presence of the chemiluminescent-tagged ***antibody*** is then determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of. . .

DRWD [0212] Likewise, a bioluminescent compound may be used to label the

antibody of the present invention. Bioluminescence is a type of chemiluminescence found in biological systems in, which a catalytic protein increases. . .

DRWD . . . a receptor with which SSP-1 is capable of interacting.

Preferred compounds which are capable of interacting with SSP-1 include anti-SSP-1 ***antibodies*** and derivatives thereof, as well as soluble forms of an SSP-1 binding partner, such as a soluble SSP-1 receptor. A. . .

DRWD . . . and thereby degrades or causes the SSP-1 protein to be degraded. For example, such an SSP-1 therapeutic can be an ***antibody*** or derivative thereof which interacts specifically with an SSP-1 protein (either wild-type or mutated).

DRWD . . . (e.g., biotinylation kit, Pierce Chemicals, Rockford, Ill.), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, ***antibodies*** reactive with SSP-1 can be derivatized to the wells of the plate, and SSP-1 trapped in the wells by ***antibody*** conjugation. As above, preparations of a SSP-1 binding protein and a test compound are incubated in the SSP-1 presenting wells. . . methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using ***antibodies*** reactive with the SSP-1 binding partner, or which are reactive with SSP-1 protein and compete with the binding partner; as. . .

DRWD [0229] For processes which rely on immunodetection for quantitating one of the proteins trapped in the complex, ***antibodies*** against the protein, such as anti-SSP-1 ***antibodies***, can be used.

Alternatively, the protein to be detected in the complex can be "epitope tagged" in the form of a fusion protein which includes, in addition to the SSP-1 sequence, a second polypeptide for which ***antibodies*** are readily available (e.g. from commercial sources). For instance, the GST fusion proteins described above can also be used for quantification of binding using ***antibodies*** against the GST moiety. Other useful epitope tags include myc-epitopes (e.g., see Ellison et al. (1991) J Biol Chem 266:21150-21157). . .

DRWD . . . analysis, using a probe that is complementary to at least a portion of the transgene. Western blot analysis using an ***antibody*** against the protein encoded by the transgene may be employed as an alternative or additional method for screening for the.

DRWD . . . expression of the SSP-1 gene knocked out in various tissues of the offspring by probing the Western blot with an ***antibody*** against the particular SSP-1 protein, or an ***antibody*** against the marker gene product, where this gene is expressed. Finally, in situ analysis (such as fixing the cells and labeling with ***antibody***) and/or FACS (fluorescence activated cell sorting) analysis of various cells from the offspring can be conducted using suitable ***antibodies*** to look for the presence or absence of the knockout construct gene product.

DETD . . . 1984, Cell 37, 767). The infusion of HA tag to SSP-1 allows easy detection of the recombinant protein with an ***antibody*** that recognizes the HA epitope.

DETD . . . Press, (1989)). The expression of the SSP-1 -HA protein can be detected by radiolabelling and immunoprecipitation. (E. Harlow, D. Lane, ***Antibodies*** : A Laboratory Manual, Cold Spring Harbor Laboratory Press, (1988)). For this, transfected cells are labelled with

.sup.35S-cysteine two days post transfection. Culture media is then collected and the SSP-1 protein immunoprecipitated with an HA specific monoclonal ***antibody***. Proteins precipitated can then be analyzed on SDS-PAGE gels.

L9 ANSWER 9 OF 9 USPATFULL on STN
AN 2000:124823 USPATFULL
TI Human Delta3 nucleic acid molecules
IN ***McCarthy, Sean Anthony***, Boston, MA, United States
Gearing, David Paul, Wellesley, MA, United States
PA Millennium Biotherapeutics, Inc., Cambridge, MA, United States (U.S. corporation)
PI US 6121045 20000919
AI US 1997-872855 19970611 (8)
RLI Continuation-in-part of Ser. No. US 1997-832633, filed on 4 Apr 1997, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Duffy, Patricia A.
LREP Foley, Hoag & Eliot, LLP, Arnold, Esq., Beth E., Clauss, Ph.D., Isabelle M.
CLMN Number of Claims: 21
ECL Exemplary Claim: 1
DRWN 5 Drawing Figure(s); 9 Drawing Page(s)
LN.CNT 5656
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides nucleic acid molecules which encode polypeptides having homology to proteins in the Delta family of proteins. The invention also provides vectors containing nucleic acid molecules of the invention and host cells containing the vectors.
IN ***McCarthy, Sean Anthony***, Boston, MA, United States
SUMM . . . preparation, the immunogen being capable of eliciting an immune response specific for a Delta3 polypeptide, e.g. a humoral response, an ***antibody*** response and/or cellular response. In preferred embodiments, the immunogen comprises an antigenic determinant, e.g. a unique determinant, from the protein. . .
SUMM A still further aspect of the present invention features ***antibodies*** and ***antibody*** preparations specifically reactive with an epitope of the Delta3 protein. In preferred embodiments, the ***antibody*** specifically binds to an epitope represented in SEQ ID No: 2.
SUMM In alternate embodiments, the diagnostic methods comprise determining the level of a Delta3 protein in an immunoassay using an ***antibody*** which is specifically immunoreactive with a wildtype or mutant Delta3 protein.
DETD Accordingly, certain aspects of the present invention relate to Delta3 proteins, nucleic acid molecules encoding Delta3 proteins, ***antibodies*** immunoreactive with Delta3 proteins, and preparations of such compositions. In addition, drug discovery assays are provided for identifying agents that. . .
DETD . . . presently known or inherent. Antigenic functions include possession of an epitope or antigenic site that is capable of cross-reacting with ***antibodies*** raised against a naturally occurring or denatured Delta3 polypeptide or fragment thereof. Accordingly, a biological activity of a Delta3 protein. . .

DETD . . . intact tissue and tissue samples for the presence (or absence) of Delta-encoding transcripts. Similar to the diagnostic uses of anti-Delta3 ***antibodies***, the use of probes directed to Delta3 messages, or to genomic Delta3 sequences, can be used for both predictive and. . .

DETD . . . into the tissue site, or modified antisense molecules, designed to target the desired cells e.g., antisense linked to peptides or ***antibodies*** that specifically bind receptors or antigens expressed on the target cell surface) can be administered systematically.

DETD . . . techniques known in the art for purifying proteins including ion-exchange chromatography, gel filtration chromatography, ultrafiltration, electrophoresis, and immunoaffinity purification with ***antibodies*** specific for such peptide. In a preferred embodiment, the recombinant Delta3 polypeptide is a fusion protein containing a domain which. . .

DETD . . . form of a viral particle. The nucleic acid sequences corresponding to the portion of a subject Delta3 protein to which ***antibodies*** are to be raised can be incorporated into a fusion gene construct which includes coding sequences for a late vaccinia. .

DETD 4.4.3. ***Antibodies***

DETD Another aspect of the invention pertains to an ***antibody*** specifically reactive with a Delta3 protein. For example, by using immunogens derived from a Delta3 protein, e.g. based on the cDNA sequences, anti-protein/anti-peptide antisera or monoclonal ***antibodies*** can be made by standard protocols (See, for example, ***Antibodies*** : A Laboratory Manual ed. by Harlow and Lane (Cold Spring Harbor Press: 1988)). A mammal, such as a mouse, a. . . an immunogenic form of the peptide (e.g., a Delta3 polypeptide or an antigenic fragment which is capable of eliciting an ***antibody*** response, or a fusion protein as described above). Techniques for conferring immunogenicity on a protein or peptide include conjugation to. . . Delta3 protein can be administered in the presence of adjuvant. The progress of immunization can be monitored by detection of ***antibody*** titers in plasma or serum. Standard ELISA or other immunoassays can be used with the immunogen as antigen to assess the levels of ***antibodies***. In a preferred embodiment, the subject ***antibodies*** are immunospecific for antigenic determinants of a Delta3 protein of a mammal, e.g. antigenic determinants of a protein represented by. . .

DETD . . . an animal with an antigenic preparation of a Delta3 polypeptide, anti-Delta3 antisera can be obtained and, if desired, polyclonal anti-Delta3 ***antibodies*** isolated from the serum. To produce monoclonal ***antibodies***, ***antibody***-producing cells (lymphocytes) can be harvested from an immunized animal and fused by standard somatic cell fusion procedures with immortalizing cells. . . B cell hybridoma technique (Kozbar et al., (1983) Immunology Today, 4: 72), and the EBV-hybridoma technique to produce human monoclonal ***antibodies*** (Cole et al., (1985) Monoclonal ***Antibodies*** and Cancer Therapy, Alan R. Liss, Inc. pp. 77-96). Hybridoma cells can be screened immunochemically for production of ***antibodies*** specifically reactive with a Delta3 polypeptide of the present invention and monoclonal ***antibodies*** isolated from a culture comprising such hybridoma cells. In one embodiment anti-human Delta3

antibodies specifically react with the proteins encoded by the DNA of ATCC Deposit Accession Number 98348.

DETD The term ***antibody*** as used herein is intended to include fragments thereof which are also specifically reactive with one of the subject Delta3 polypeptides. ***Antibodies*** can be fragmented using conventional techniques and the fragments screened for utility in the same manner as described above for whole ***antibodies***. For example, F(ab).sub.2 fragments can be generated by treating ***antibody*** with pepsin. The resulting F(ab).sub.2 fragment can be treated to reduce disulfide bridges to produce Fab fragments. The ***antibody*** of the present invention is further intended to include bispecific and chimeric molecules having affinity for a Delta3 protein conferred by at least one CDR region of the ***antibody***.

DETD ***Antibodies*** which specifically bind Delta3 epitopes can also be used in immunohistochemical staining of tissue samples in order to evaluate the abundance and pattern of expression of each of the subject Delta3 polypeptides. Anti-Delta3 ***antibodies*** can be used diagnostically in immuno-precipitation and immuno-blotting to detect and evaluate Delta3 protein levels in tissue as part of. . . spinal fluid or amniotic fluid, or can be measured in tissue, such as produced by biopsy. Diagnostic assays using anti-Delta3 ***antibodies*** can include, for example, immunoassays designed to aid in early diagnosis of a neurodegenerative disorder, particularly ones which are manifest at birth. Diagnostic assays using anti-Delta3 polypeptide ***antibodies*** can also include immunoassays designed to aid in early diagnosis and phenotyping neurodegenerative, neoplastic or hyperplastic disorders.

DETD Another application of anti-Delta3 ***antibodies*** of the present invention is in the immunological screening of cDNA libraries constructed in expression vectors such as .lambda.gt11, .lambda.gt18-23,. . . e.g. other orthologs of a particular Delta3 protein or other paralogs from the same species, can then be detected with ***antibodies***, as, for example, reacting nitrocellulose filters lifted from infected plates with anti-Delta3 ***antibodies***. Positive phage detected by this assay can then be isolated from the infected plate. Thus, the presence of Delta3 homologs. . .

DETD . . . of childhood (Fazio-Londe syndrome), poliomyelitis, and hereditary motorsensory neuropathy (Charcot-Marie-Tooth disease), spinal cord injuries, brain injuries, lesions associated with surgery, ***ischemic*** lesions, malignant lesions, infectious lesions.

DETD . . . tumors. Alternatively, cells expressing or overexpressing Delta3 can be targeted for lysis, by, for example, targeting to the cell an ***antibody*** binding specifically to a Delta3 protein linked to a cytotoxic peptide.

DETD . . . described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or ***antibody*** reagent described herein, which may be conveniently used, e.g., in clinical settings to diagnose patients exhibiting symptoms or family history. . .

DETD ***Antibodies*** directed against wild type or mutant Delta3 proteins, which are discussed, above, may also be used in disease diagnostics and. . . herein may also be such as those described in Harlow and Lane, for example, (Harlow, E. and Lane, D., 1988, " ***Antibodies*** : A Laboratory Manual", Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York), which is incorporated herein by

reference in. . .

DETD This can be accomplished, for example, by immunofluorescence techniques employing a fluorescently labeled ***antibody*** (see below) coupled with light microscopic, flow cytometric, or fluorimetric detection. The ***antibodies*** (or fragments thereof) useful in the present invention may, additionally, be employed histologically, as in immunofluorescence or immunoelectron microscopy, for. . . proteins. In situ detection may be accomplished by removing a histological specimen from a patient, and applying thereto a labeled ***antibody*** of the present invention. The ***antibody*** (or fragment) is preferably applied by overlaying the labeled ***antibody*** (or fragment) onto a biological sample. Through the use of such a procedure, it is possible to determine not only. . .

DETD Often a solid phase support or carrier is used as a support capable of binding an antigen or an ***antibody***. Well-known supports or carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, gabbros, and magnetite.. . . have virtually any possible structural configuration so long as the coupled molecule is capable of binding to an antigen or ***antibody***. Thus, the support configuration may be spherical, as in a bead, or cylindrical, as in the inside surface of a. . . strip, etc. Preferred supports include polystyrene beads. Those skilled in the art will know many other suitable carriers for binding ***antibody*** or antigen, or will be able to ascertain the same by use of routine experimentation.

DETD One means for labeling an anti-Delta3 protein specific ***antibody*** is via linkage to an enzyme and use in an enzyme immunoassay (EIA) (Voller, "The Enzyme Linked Immunosorbent Assay (ELISA)",. . . Raton, Fla., 1980; Ishikawa, et al., (eds.) Enzyme Immunoassay, Kagaku Shoin, Tokyo, 1981). The enzyme which is bound to the ***antibody*** will react with an appropriate substrate, preferably a chromogenic substrate, in such a manner as to produce a chemical moiety. . . be detected, for example, by spectrophotometric, fluorimetric or by visual means. Enzymes which can be used to detectably label the ***antibody*** include, but are not limited to, malate dehydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, yeast alcohol dehydrogenase, alpha-glycerophosphate, dehydrogenase, triose phosphate isomerase,. . .

DETD Detection may also be accomplished using any of a variety of other immunoassays. For example, by radioactively labeling the ***antibodies*** or ***antibody*** fragments, it is possible to detect fingerprint gene wild type or mutant peptides through the use of a radioimmunoassay (RIA). . .

DETD It is also possible to label the ***antibody*** with a fluorescent compound. When the fluorescently labeled ***antibody*** is exposed to light of the proper wave length, its presence can then be detected due to fluorescence. Among the. . .

DETD The ***antibody*** can also be detectably labeled using fluorescence emitting metals such as .sup.152 Eu, or others of the lanthanide series. These metals can be attached to the ***antibody*** using such metal chelating groups as diethylenetriaminepentacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA).

DETD The ***antibody*** also can be detectably labeled by coupling it to a chemiluminescent compound. The presence of the chemiluminescent-tagged ***antibody*** is then determined by detecting the presence of

luminescence that arises during the course of a chemical reaction.

Examples of. . .

DETD Likewise, a bioluminescent compound may be used to label the ***antibody*** of the present invention. Bioluminescence is a type of chemiluminescence found in biological systems in, which a catalytic protein increases. . .

DETD . . . (e.g., biotinylation kit, Pierce Chemicals, Rockford, Ill.), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, ***antibodies*** reactive with Delta3 but which do not interfere with binding of upstream or downstream elements can be derivatized to the wells of the plate, and Delta3 trapped in the wells by ***antibody*** conjugation. As above, preparations of a Delta-binding protein and a test compound are incubated in the Delta-presenting wells of the. . . methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using ***antibodies*** reactive with the Delta3 binding element, or which are reactive with Delta3 protein and compete with the binding element; as. . .

DETD For processes which rely on immunodetection for quantitating one of the proteins trapped in the complex, ***antibodies*** against the protein, such as anti-Delta3 ***antibodies***, can be used. Alternatively, the protein to be detected in the complex can be "epitope tagged" in the form of a fusion protein which includes, in addition to the Delta3 sequence, a second polypeptide for which ***antibodies*** are readily available (e.g. from commercial sources). For instance, the GST fusion proteins described above can also be used for quantification of binding using ***antibodies*** against the GST moiety. Other useful epitope tags include myc-epitopes (e.g., see Ellison et al. (1991) J Biol Chem 266:21150-21157). . .

DETD . . . analysis, using a probe that is complementary to at least a portion of the transgene. Western blot analysis using an ***antibody*** against the protein encoded by the transgene may be employed as an alternative or additional method for screening for the. . .

DETD . . . expression of the Delta3 gene knocked out in various tissues of the offspring by probing the Western blot with an ***antibody*** against the particular Delta3 protein, or an ***antibody*** against the marker gene product, where this gene is expressed. Finally, in situ analysis (such as fixing the cells and labeling with ***antibody***) and/or FACS (fluorescence activated cell sorting) analysis of various cells from the offspring can be conducted using suitable ***antibodies*** to look for the presence or absence of the knockout construct gene product.

DETD . . . surface and the other protein is labeled, e.g., such as by tagging the protein with an epitope, for which an ***antibody*** is available (e.g., FLAG epitope, available from International Biotechnologies, Inc.). For example, the Delta3 protein can be linked to the. . . wells is determined. The amount of bound protein can be determined by incubating the wells with an anti-tag, e.g, anti-myc, ***antibody***, which can then be detected by enzyme immunoassay. The amount of bound protein is then determined by determining the optical.

*****STN Columbus*****

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=> s antibod? and ischem? and (200 gene product?)

L1 7 ANTIBOD? AND ISCHEM? AND (200 GENE PRODUCT?)

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L2 7 DUP REM L1 (0 DUPLICATES REMOVED)

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YOU HAVE REQUESTED DATA FROM 7 ANSWERS - CONTINUE? Y/(N):y

L2 ANSWER 1 OF 7 USPATFULL on STN

AN 2003:187823 USPATFULL

TI Novel human proteins and polynucleotides encoding them

IN Fernandes, Elma R., Branford, CT, UNITED STATES

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PI US 2003129613 A1 20030710

AI US 2002-189940 A1 20020703 (10)

RLI Continuation-in-part of Ser. No. US 2001-965212, filed on 26 Sep 2001,
PENDING Continuation-in-part of Ser. No. US 2001-966545, filed on 26 Sep
2001, PENDING Continuation-in-part of Ser. No. US 2001-966546, filed on
26 Sep 2001, PENDING

PRAI US 2001-303241P 20010705 (60)

US 2002-369065P 20020401 (60)

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US 2002-384327P 20020530 (60)

DT Utility

FS APPLICATION

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CLMN Number of Claims: 71

ECL Exemplary Claim: 1

DRWN 75 Drawing Page(s)

LN.CNT 5672

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention provides novel isolated SECX polynucleotides and
the membrane-associated or secreted polypeptides encoded by the SECX
polynucleotides. Also provided are the ***antibodies*** that
immunospecifically bind to a SECX polypeptide or any derivative,
variant, mutant or fragment of the SECX polypeptide, polynucleotide or
antibody. The invention additionally provides methods in which
the SECX polypeptide, polynucleotide and ***antibody*** are utilized
in the detection and treatment of a broad range of pathological states,
as well as to other uses.

AB . . . provides novel isolated SECX polynucleotides and the
membrane-associated or secreted polypeptides encoded by the SECX
polynucleotides. Also provided are the ***antibodies*** that

immunospecifically bind to a SECX polypeptide or any derivative, variant, mutant or fragment of the SECX polypeptide, polynucleotide or ***antibody***. The invention additionally provides methods in which the SECX polypeptide, polynucleotide and ***antibody*** are utilized in the detection and treatment of a broad range of pathological states, as well as to other uses.

SUMM [0002] The invention relates to polynucleotides and polypeptides encoded by such polynucleotides, as well as vectors, host cells, ***antibodies*** and recombinant methods for producing the polypeptides and polynucleotides.

SUMM [0011] Also included in the invention is an ***antibody*** which selectively binds to a SECX polypeptide.

SUMM . . . nucleic acid in a sample from a mammal, e.g., a human, by contacting a sample from the mammal with an ***antibody*** which selectively binds to one of the herein described polypeptides, and detecting the formation of reaction complexes including the ***antibody*** and the polypeptide in the sample. Detecting the formation of complexes in the sample indicates the presence of the polypeptide in the sample. Methods for measurements of ***antibody*** reaction complex concentrations are well known in the art. Methods for detecting and quantitating nucleic acids include hybridization and TaqMan.TM.. . .

SUMM [0018] Alternatively, the mammal may be treated by administering an ***antibody*** as herein described in an amount sufficient to alleviate the pathological condition.

DETD . . . having 180 residues (WO9932632-A1, published Jul. 1, 1999). The EDIRF-like DNA and protein sequences (e.g., Clone 2191999) and their homologues, ***antibodies*** (Ab) specific for EDIRF-like protein, and other modulators may be used: (i) in screening and detection assays, e.g. for chromosome. . .

DETD . . . fragment of human limbic system associated membrane protein (LAMP; PCT Publication WO9630052-A1, published Oct. 3, 1996). LAMP is a self-binding, ***antibody***-like cell surface adhesion protein involved in formation of connections between adjacent neurons. LAMP protein, and by analogy the clone 4324229. . .

DETD . . . Ig superfamily class. Expression of 200 gene is many-fold higher in TH1 than in TH2 subpopulations (WO9627603-A1). Modulation of the ***200*** ***gene*** ***product*** may ameliorate a range of T-cell-related disorders. BLASTP searches also show a moderate degree of similarity to kidney injury molecule-1. . .

DETD . . . (ACC:P55083 and U.S. Pat. No. 5972654-A, issued Oct. 26, 1999). The human microfibril-associated glycoprotein 4 splice variant (MAG4V) polypeptides and/or ***antibodies*** thereto are disclosed in this patent as being usable to down regulate MAG4V expression and activity. By analogy, Clone 4437909.0.4. . .

DETD . . . as a diagnostic or prognostic marker for the diseases indicated herein. Potential therapeutic applications include protein therapeutics, small molecule targets, ***antibody*** targets, nucleic acids useful in gene therapy, and the like.

DETD . . . receptors or antigens expressed on a selected cell surface, e.g., by linking the antisense nucleic acid molecules to peptides or ***antibodies*** that bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using.

DETD . . . or derivatives, fragments, analogs or homologs thereof. Also

provided are polypeptide fragments suitable for use as immunogens to raise anti-SECX ***antibodies***. In one embodiment, native SECX proteins can be isolated from cells or tissue sources by an appropriate purification scheme using. . .

DETD . . . or inhibiting) cell survival. Moreover, the SECX-immunoglobulin fusion proteins of the invention can be used as immunogens to produce anti-SECX ***antibodies*** in a subject, to purify SECX ligands, and in screening assays to identify molecules that inhibit the interaction of SECX. . .

DETD [0204] Anti-SECX ***Antibodies***

DETD [0205] The invention encompasses ***antibodies*** and ***antibody*** fragments, such as F.sub.ab or (F.sub.ab).sub.2, that bind immunospecifically to any of the polypeptides of the invention.

DETD [0206] An isolated SECX protein, or a portion or fragment thereof, can be used as an immunogen to generate ***antibodies*** that bind SECX using standard techniques for polyclonal and monoclonal ***antibody*** preparation. The full-length SECX protein can be used or, alternatively, the invention provides antigenic peptide fragments of SECX for use. . .
. 28, 30, 87, 88, 126, 127, 145, 146, 148 and 150 and encompasses an epitope of SECX such that an ***antibody*** raised against the peptide forms a specific immune complex with SECX. Preferably, the antigenic peptide comprises at least 6, 8, . . .

DETD . . . which regions of a SECX polypeptide are particularly hydrophilic and, therefore, are likely to encode surface residues useful for targeting ***antibody*** production. As a means for targeting ***antibody*** production, hydropathy plots showing regions of hydrophilicity and hydrophobicity may be generated by any method well known in the art, . . .

DETD . . . 146, 148 and 150, or derivatives, fragments, analogs or homologs thereof, may be utilized as immunogens in the generation of ***antibodies*** that immunospecifically-bind these protein components. The term " ***antibody*** " as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that specifically binds (immunoreacts with) an antigen, such as SECX. Such ***antibodies*** include, but are not limited to, polyclonal, monoclonal, chimeric, single chain, F.sub.ab and F.sub.(ab')₂ fragments, and an F.sub.ab expression library. In a specific embodiment, ***antibodies*** to human SECX proteins are disclosed. Various procedures known within the art may be used for the production of polyclonal or monoclonal ***antibodies*** to a SECX protein sequence, or derivative, fragment, analog or homolog thereof. Some of these proteins are discussed below.

DETD [0209] For the production of polyclonal ***antibodies***, various suitable host animals (e.g., rabbit, goat, mouse or other mammal) may be immunized by injection with the native protein, . . . oil emulsions, dinitrophenol, etc.), human adjuvants such as Bacille Calmette-Guerin and Corynebacterium parvum, or similar immunostimulatory agents. If desired, the ***antibody*** molecules directed against SECX can be isolated from the mammal (e.g., from the blood) and further purified by well known. . .

DETD [0210] The term "monoclonal ***antibody*** " or "monoclonal ***antibody*** composition", as used herein, refers to a population of ***antibody*** molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of

SECX. A monoclonal ***antibody*** composition thus typically displays a single binding affinity for a particular SECX protein with which it immunoreacts. For preparation of monoclonal ***antibodies*** directed towards a particular SECX protein, or derivatives, fragments, analogs or homologs thereof, any technique that provides for the production of ***antibody*** molecules by continuous cell line culture may be utilized. Such techniques include, but are not limited to, the hybridoma technique. . . hybridoma technique (see Kozbor, et al., 1983 Immunol Today 4: 72) and the EBV hybridoma technique to produce human monoclonal ***antibodies*** (see Cole, et al., 1985 In: MONOCLONAL ***ANTIBODIES*** AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96). Human monoclonal ***antibodies*** may be utilized in the practice of the present invention and may be produced by using human hybridomas (see Cote, . . . 80: 2026-2030) or by transforming human B-cells with Epstein Barr Virus in vitro (see Cole, et al., 1985 In: MONOCLONAL ***ANTIBODIES*** AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96). Each of the above citations are incorporated herein by reference in. . .

DETD [0211] According to the invention, techniques can be adapted for the production of single-chain ***antibodies*** specific to a SECX protein (see e.g., U.S. Pat. No. 4,946,778). In addition, methodologies can be adapted for the construction. . . of monoclonal F.sub.ab fragments with the desired specificity for a SECX protein or derivatives, fragments, analogs or homologs thereof. Non-human ***antibodies*** can be "humanized" by techniques well known in the art. See e.g., U.S. Pat. No. 5,225,539. ***Antibody*** fragments that contain the idiotypes to a SECX protein may be produced by techniques known in the art including, but not limited to: (i) an F.sub.(ab')₂ fragment produced by pepsin digestion of an ***antibody*** molecule; (ii) an F.sub.ab fragment generated by reducing the disulfide bridges of an F.sub.(ab')₂ fragment; (iii) an F.sub.ab fragment generated by the treatment of the ***antibody*** molecule with papain and a reducing agent and (iv) F.sub.v fragments.

DETD [0212] Additionally, recombinant anti-SECX ***antibodies***, such as chimeric and humanized monoclonal ***antibodies***, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the invention. Such chimeric and humanized monoclonal ***antibodies*** can be produced by recombinant DNA techniques known in the art, for example using methods described in International Application No. . . .

DETD [0213] In one embodiment, methodologies for the screening of ***antibodies*** that possess the desired specificity include, but are not limited to, enzyme-linked immunosorbent assay (ELISA) and other immunologically-mediated techniques known within the art. In a specific embodiment, selection of ***antibodies*** that are specific to a particular domain of a SECX protein is facilitated by generation of hybridomas that bind to the fragment of a SECX protein possessing such a domain. ***Antibodies*** that are specific for an above-described domain within a SECX protein, or derivatives, fragments, analogs or homologs thereof, are also. . .

DETD [0214] Anti-SECX ***antibodies*** may be used in methods known within the art relating to the localization and/or quantitation of a SECX protein (e.g., . . . physiological samples, for use in diagnostic methods, for use in imaging the protein, and the like). In a given embodiment, ***antibodies*** for SECX proteins, or derivatives,

fragments, analogs or homologs thereof, that contain the ***antibody*** derived binding domain, are utilized as pharmacologically-active compounds [hereinafter "Therapeutics"].

DETD [0215] An anti-SECX ***antibody*** (e.g., monoclonal ***antibody***) can be used to isolate SECX by standard techniques, such as affinity chromatography or immunoprecipitation. An anti-SECX ***antibody*** can facilitate the purification of natural SECX from cells and of recombinantly produced SECX expressed in host cells. Moreover, an anti-SECX ***antibody*** can be used to detect SECX protein (e.g., in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the SECX protein. Anti-SECX ***antibodies*** can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, e.g., to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (i.e., physically linking) the ***antibody*** to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and. . .

DETD [0242] The SECX nucleic acid molecules, SECX proteins, and anti-SECX ***antibodies*** (also referred to herein as "active compounds") of the invention, and derivatives, fragments, analogs and homologs thereof, can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the nucleic acid molecule, protein, or ***antibody*** and a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion. . .

DETD [0245] Sterile injectable solutions can be prepared by incorporating the active compound (e.g., a SECX protein or anti-SECX ***antibody***) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed. .

DETD . . . be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal ***antibodies*** to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to. . .

DETD [0255] The nucleic acid molecules, proteins, protein homologues, and ***antibodies*** described herein that include extracellular and transmembrane domains and, therefore, can be used in one or more of the following. . .

DETD . . . disorders such as cancer or preclampsia, or any disease or disorder described in Sections 1-14 above). In addition, the anti-SECX ***antibodies*** of the invention can be used to detect and isolate SECX proteins and modulate SECX activity.

DETD . . . (e.g., biotinylation kit, Pierce Chemicals, Rockford, Ill.), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, ***antibodies*** reactive with SECX or target molecules, but which do not interfere with binding of the SECX protein to its target. . . molecule, can be derivatized to the wells of the plate, and unbound target or SECX trapped in the wells by ***antibody*** conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using ***antibodies*** reactive with the SECX or target molecule, as well as enzyme-linked assays that rely on detecting an enzymatic activity associated. . .

- DETD . . . been implicated in cardiovascular disorders, including in atherosclerotic plaque formation. Diseases such as cardiovascular disease, including cerebral thrombosis or hemorrhage, ***ischemic*** heart or renal disease, peripheral vascular disease, or thrombosis of other major vessel, and other diseases, including diabetes mellitus, hypertension,. . .
- DETD . . . with a monomeric form of a peptide having an activity of another B lymphocyte antigen (e.g., B7-1, B7-3) or blocking ***antibody***), prior to transplantation can lead to the binding of the molecule to the natural ligand(s) on the immune cells without. . .
- DETD . . . of inappropriate activation of T cells that are reactive against self tissue and which promote the production of cytokines and auto- ***antibodies*** involved in the pathology of the diseases. Preventing the activation of autoreactive T cells may reduce or eliminate disease symptoms.. . . by disrupting receptor:ligand interactions of B lymphocyte antigens can be used to inhibit T cell activation and prevent production of auto- ***antibodies*** or T cell-derived cytokines which may be involved in the disease process. Additionally, blocking reagents may induce antigen-specific tolerance of. . .
- DETD [0342] Assays for T-cell-dependent immunoglobulin responses and isotype switching (which will identify, among others, proteins that modulate T-cell dependent ***antibody*** responses and that affect Th1/Th2 profiles) include, without limitation, those described in: Maliszewski, J Immunol 144:3028-3033,1990; and Mond and Brunswick. . .
- DETD . . . acute conditions), including without limitation inflammation associated with infection (such as septic shock, sepsis or systemic inflammatory response syndrome (SIRS)), ***ischemia*** -reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine-induced lung injury, inflammatory bowel disease, Crohn's disease or resulting. . .
- DETD . . . Methods include, e.g., Northern or Southern blot hybridization (above) or quantitative PCR (below) for measuring SECX nucleic acid levels, and ***antibody*** detection (below), such as ELISA assays and ***antibody*** pull-down assay for measuring SECX polypeptide levels.
- DETD [0391] An agent for detecting SECX protein is an ***antibody*** capable of binding to SECX protein, preferably an ***antibody*** with a detectable label. ***Antibodies*** can be polyclonal, or more preferably, monoclonal. An intact ***antibody***, or a fragment thereof (e.g., Fab or F(ab').sub.2) can be used. The term "labeled", with regard to the probe or ***antibody***, is intended to encompass direct labeling of the probe or ***antibody*** by coupling (i.e., physically linking) a detectable substance to the probe or ***antibody***, as well as indirect labeling of the probe or ***antibody*** by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary ***antibody*** using a fluorescently labeled secondary ***antibody*** and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin. The term. . . . include Southern hybridizations. Furthermore, in vivo techniques for detection of SECX protein include introducing into a subject a labeled anti-SECX ***antibody***. For example, the ***antibody*** can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging. . .

DETD . . . described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or ***antibody*** reagent described herein, which may be conveniently used, e.g., in clinical settings to diagnose patients exhibiting symptoms or family history. . .

DETD . . . be utilized include, but are not limited to, (i) an aforementioned peptide, or analogs, derivatives, fragments or homologs thereof; (ii) ***antibodies*** to an aforementioned peptide; (iii) nucleic acids encoding an aforementioned peptide; (iv) administration of antisense nucleic acid and nucleic acids. . . 1989, Science 244: 1288-1292); or (v) modulators (i.e., inhibitors, agonists and antagonists, including additional peptide mimetic of the invention or ***antibodies*** specific to a peptide of the invention) that alter the interaction between an aforementioned peptide and its binding partner.

DETD . . . inhibits one or more SECX protein activity. Examples of such inhibitory agents include antisense SECX nucleic acid molecules and anti-SECX ***antibodies***. These modulatory methods can be performed in vitro (e.g., by culturing the cell with the agent) or, alternatively, in vivo. . .

DETD . . . supernatant were harvested 72 hours after transfection and examined for h11753149 expression by Western blotting (reducing conditions) with an anti-V5 ***antibody***. FIG. 15 shows that h11753149 is expressed as a protein secreted by 293 cells that is broadly distributed around 64. . .

DETD . . . supernatant were harvested 72 hours after transfection and examined for h4437909 expression by Western blotting (reducing conditions) with an anti-V5 ***antibody***. FIG. 16 shows that h4437909 is expressed in 293 cells as three discrete secreted protein bands of 16, 40, and. . .

DETD . . . according to the manufacturer's instructions. After induction, total cells were harvested, and proteins were analyzed by Western blotting using anti-HisGly ***antibody*** (Invitrogen, Carlsbad, Calif.). FIG. 17 shows h4437909 was expressed as a 34 kDa protein in E. coli cells. This corresponds. . .

DETD . . . that therapeutic indications for targeting 4324229 include selected lung, breast, bladder and ovarian carcinomas. For example, therapeutic targeting with an ***antibody*** or domain thereof will block the migration and growth of cancer cells and promote cell death in cells where the. . .

CLM What is claimed is:

21. An ***antibody*** that binds immunospecifically to the polypeptide of claim 1.

22. The ***antibody*** of claim 21, wherein said ***antibody*** is a monoclonal ***antibody***.

23. The ***antibody*** of claim 21, wherein the ***antibody*** is a humanized ***antibody***.

24. An ***antibody*** that binds immunospecifically to the polypeptide of claim 5.

25. The ***antibody*** of claim 24, wherein said ***antibody*** is a monoclonal ***antibody***.

26. The ***antibody*** of claim 24, wherein the ***antibody*** is a humanized ***antibody***.

27. A method for determining the presence or amount of the polypeptide of claim 1 in a sample, the method comprising: (a) providing the sample; (b) contacting the sample with an ***antibody*** that binds immunospecifically to a polypeptide of claim 1; and (c) determining the presence or amount of ***antibody*** bound to said polypeptide, thereby determining the presence or amount of polypeptide in said sample.

. . . polypeptide of claim 5 in a sample, the method comprising: (a) providing the sample; (b) contacting the sample with an ***antibody*** that binds immunospecifically to a polypeptide of claim 5; and (c) determining the presence or amount of ***antibody*** bound to said polypeptide, thereby determining the presence or amount of polypeptide in said sample.

. . . preventing a SECX-associated disorder, said method comprising administering to a subject in which such treatment or prevention is desired the ***antibody*** of claim 21 in an amount sufficient to treat or prevent said SECX-associated disorder in said subject.

51. A pharmaceutical composition comprising the ***antibody*** of claim 21 and a pharmaceutically acceptable carrier.

52. A pharmaceutical composition comprising the ***antibody*** of claim 24 and a pharmaceutically acceptable carrier.

. . . wherein said therapeutic is selected from the group consisting of a SECX polypeptide, a SECX nucleic acid, and a SECX ***antibody***.

70. A method of treating a pathological state in a mammal, the method comprising administering to the mammal the ***antibody*** of claim 21 in an amount sufficient to alleviate the pathological state.

71. A method of treating a pathological state in a mammal, the method comprising administering to the mammal the ***antibody*** of claim 24 in an amount sufficient to alleviate the pathological state.

L2 ANSWER 2 OF 7 USPATFULL on STN

AN 2003:100085 USPATFULL

TI Compositions and methods for the treatment and diagnosis of immune disorders

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PI US 2003069196 A1 20030410

AI US 2001-4633 A1 20011204 (10)

RLI Continuation of Ser. No. US 1999-324986, filed on 2 Jun 1999, ABANDONED

Continuation-in-part of Ser. No. US 1998-32337, filed on 27 Feb 1998,

GRANTED, Pat. No. US 6455685 Continuation-in-part of Ser. No. US

1996-609583, filed on 1 Mar 1996, GRANTED, Pat. No. US 6204371
Continuation-in-part of Ser. No. US 1995-487748, filed on 7 Jun 1995,
GRANTED, Pat. No. US 5721351 Continuation-in-part of Ser. No. US
1995-398633, filed on 3 Mar 1995, GRANTED, Pat. No. US 6066322

DT Utility

FS APPLICATION

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CLMN Number of Claims: 21

ECL Exemplary Claim: 1

DRWN 47 Drawing Page(s)

LN.CNT 8502

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to methods and compositions for the treatment and diagnosis of immune disorders, especially T helper lymphocyte-related disorders, and also for the treatment of mast cell-related processes and disorders, ***ischemic*** disorders and injuries, including ***ischemic*** renal disorders and injuries. For example, genes which are differentially expressed within and among T helper (TH) cells and TH cell subpopulations, which include, but are not limited to TH0, TH1 and TH2 cell subpopulations are identified. Genes are also identified via the ability of their gene products to interact with gene products involved in the differentiation, maintenance and effector function of such TH cells and TH cell subpopulations. The genes identified can be used diagnostically or as targets for therapeutic intervention. In this regard, the present invention provides methods for the identification and therapeutic use of compounds as treatments of immune disorders, especially TH cell subpopulation-related disorders. Additionally, methods are provided for the diagnostic evaluation and prognosis of TH cell subpopulation-related disorders, for the identification of subjects exhibiting a predisposition to such conditions, for monitoring patients undergoing clinical evaluation for the treatment of such disorders, and for monitoring the efficacy of compounds used in clinical trials. Methods are also provided for the treatment of symptoms associated with mast cell-related processes or disorders and ***ischemic*** disorders and injuries using the genes, gene products and ***antibodies*** of the invention.

AB . . . diagnosis of immune disorders, especially T helper lymphocyte-related disorders, and also for the treatment of mast cell-related processes and disorders, ***ischemic*** disorders and injuries, including ***ischemic*** renal disorders and injuries. For example, genes which are differentially expressed within and among T helper (TH) cells and TH. . . in clinical trials. Methods are also provided for the treatment of symptoms associated with mast cell-related processes or disorders and ***ischemic*** disorders and injuries using the genes, gene products and ***antibodies*** of the invention.

SUMM . . . TH cells and TH cell subpopulations. Among the genes identified are ones involved in repair or recovery of tissue from ***ischemic*** disorders or injuries. The genes identified can be used diagnostically or as targets for therapeutic intervention. In this regard, the . . . of compounds as treatments of immune disorders, especially TH cell subpopulation-related disorders. The present invention also provides methods for treating ***ischemic*** disorders or injuries. Additionally, methods are provided for the diagnostic evaluation and prognosis of TH cell subpopulation-related disorders, for the . . .

SUMM . . . TH cells are involved in both humoral and cell-mediated forms of effector immune responses. With respect to the humoral, or ***antibody***, immune response, ***antibodies*** are produced by B lymphocytes through interactions with TH cells. Specifically, extracellular antigens are endocytosed by antigen-presenting cells (APCs), processed, . . . complex (MHC) molecules to CD4^{sup.}+ class II MHC-restricted TH cells. These TH cells in turn activate B lymphocytes, resulting in ***antibody*** production.

SUMM . . . cells drive humoral immune responses, which are associated with, for example, defenses against certain helminthic parasites, and are involved in ***antibody*** and allergic responses.

SUMM . . . cell and TH cell-like related disorders. The present invention additionally relates to methods and compositions for treating, ameliorating or modulating ***ischemic*** disorders or injuries or mast cell-related processes or disorders.

SUMM . . . and/or pathway gene product including, but not limited to a human and murine 10, 54, 57, 105, 106, 161 and ***200*** ***gene*** ***product***; (b) nucleotides that encode portions of a differentially expressed and/or pathway gene product that corresponds to its functional domains, and. . .

SUMM [0026] The present invention also includes the products of such fingerprint, target, and pathway genes, as well as ***antibodies*** to such gene products. Furthermore, the engineering and use of cell- and animal-based models of TH cell subpopulation-related disorders to. . .

SUMM [0029] ***Ischemic*** disorder or injury can be treated via the methods of the invention. " ***Ischemic*** disorder or injury" refers to any disorder or injury to tissues or organs which results from local deficiency of the. . . supply and/or hypoxia are generally produced by a restriction or obstruction of the blood supply to said tissue or organs. ***Ischemic*** disorders or injuries which may be treated by the methods of the present invention include, but are by no means limited to, ***ischemic*** renal disease or injury, or myocardial ***ischemia*** such as angina pectoris. ***Ischemic*** disorders or injuries which may be treated by the methods of the present invention also include damage or injury to. . . intestine, lung, and testes. The methods of the invention may also be used to regulate the extent or degree of ***ischemic*** injury in other tissues, such as tumor tissues including, but not limited to, tumors of the uterus and ovaries. The ***ischemic*** disorders or injuries which may be treated by the methods of the present invention still further include ***ischemic*** injury or damage to transplanted organs which occurs during transplant.

SUMM . . . alternative embodiment of the present invention, the methods a compositions described herein can also be used in the treatment of ***ischemic*** disorders or injuries. For example, presented herein are methods of using the 200 gene, its gene product, and ***antibodies*** thereto to treat or regulate ***ischemic*** disorders and/or injuries. In particular, the genes or gene products of the invention may be administered to an individual so as to ameliorate the symptoms of the ***ischemic*** disorder or injury. Further, compounds, such as specific ***antibodies***, including monoclonal ***antibodies***, which bind specifically to the genes or gene products of the present invention and modulate their expression or activity, may also be administered to an individual suffering from an ***ischemic*** disorder or injury.

SUMM . . . of 10 gene product activity, can be particularly amenable to

modulation. For example, natural ligands, derivatives of natural ligands and ***antibodies*** which bind to the 10 gene product can be utilized to reduce the number of induced T cells present by. . .

SUMM . . . product, including, for example, soluble fusion proteins such as Ig-tailed fusion proteins, can be particularly useful for this purpose. Additionally, ***antibodies*** directed against one or ore of the extracellular portions of the 10 gene product may either reduce 10 gene product function by, for example, locking ligand binding. Additionally, ***antibodies*** directed against the 10 gene product can, in certain instances, serve to increase the level of 10 gene product activity.

SUMM . . . IgE immune response, including but not limited to the symptoms which accompany atopic conditions such as allergy and/or asthma. IgE-type ***antibodies*** are produced by stimulated B cells which require, at least in part, IL-4 produced by the TH2 cell subpopulation. Therefore,. . .

SUMM . . . one of its gene products is a membrane-bound TH2 cell subpopulation molecule. Accordingly, natural ligands, derivatives of natural ligands and ***antibodies*** which bind to this 103 gene product, can be utilized to reduce the number of TH2 cells present by either. . . for example, soluble fusion proteins such as Ig-tailed fusion proteins, can be particularly useful for this purpose. In certain instances, ***antibodies*** directed against the 103 gene product, such as directed against the extracellular domain of the 103 gene product, can be. . .

SUMM [0054] The ***200*** ***gene*** ***product*** can be particularly suitable for such a purpose in that it is not only TH1 cell subpopulation-restricted, but the Ig superfamily ***200*** ***gene*** ***product*** is, additionally, membrane-bound. Therefore, natural ligands, derivatives of natural ligands and ***antibodies*** which bind to the ***200*** ***gene*** ***product*** can be utilized to reduce the number of TH1 cells present by either physically separating such cells away from other. . . inhibiting the proliferation of such TH1 cells. Additionally, compounds such as 200 gene sequences or gene products such as soluble ***200*** ***gene*** ***products***, can be utilized to reduce the level of TH2 cell activity, thus bringing about the amelioration of TH1 cell subpopulation-related disorders. For example, the compounds can compete with the endogenous (i.e., natural) ligand for the ***200*** ***gene*** ***product***. The resulting reduction in the amount of ligand-bound 200 gene transmembrane protein will modulate TH2 cellular activity. Soluble proteins or. . . as peptides comprising the extracellular domain, or portions (such as, for example, the Ig portion) and/or analogs thereof, of the ***200*** ***gene*** ***product***, including, for example, soluble fusion proteins such as Ig-tailed fusion proteins, can be particularly useful for this purpose. The Example presented in Section 10, below, describes the construction and expression of ***200*** ***gene*** ***product*** and 103 gene product Ig fusion constructs and proteins.

SUMM [0055] Further, the Example presented in Section 12, below, describes successful use of ***antibodies*** directed against the 103 gene product as well as 103/Ig fusion proteins to ameliorate symptoms of asthma in an accepted. . .

SUMM . . . gene products described herein are ones also involved in processes related to tissue repair and remodeling after injury,

particularly after ***ischemic*** injury. In particular, the example presented in Section 13, below, demonstrates the successful use of ***antibodies*** which bind to the extracellular domain of the ***200*** ***gene*** ***product*** to inhibit repair of ***ischemic*** kidney injury. Thus, the invention also makes possible the treatment of ***ischemic*** disorders and injuries. The invention is also based, in part, on the discovery that the 103 gene is expressed in. . .

SUMM [0066] " ***Ischemic*** disorder or injury", as used herein, refers to any disorder or injury to tissues or organs which results from local.

SUMM [0067] The term "mast-cell related processes or disorders", as used herein, includes, but is not limited to, atherosclerosis and myocardial ***ischemia*** /reperfusion.

DRWD . . . D10.G4, DAX; TH1: AE7.A, Dorris, D1.1). Clones were either unstimulated (-) or stimulated (+) for 6 hours with plate-bound anti-CD3 ***antibody***. Ten micrograms of total RNA were loaded per lane. The positions of 18s and 28s ribosomal RNA are shown as. . .

DRWD . . . 6, brain; lane 7, heart; lane 8, lung; lane 9, spleen; lane 10, liver. Clones were stimulated with plate-bound anti-CD3 ***antibody*** for 6 hours. 7.5 and 10 micrograms total RNA was used for each cell line and each tissue, respectively. a,. . .

DRWD . . . of the full length murine 200 gene. Bottom line: murine 200 gene nucleotide sequence (SEQ ID NO:8); top line: murine ***200*** ***gene*** ***product*** derived amino acid sequence (SEQ ID NO: 10).

DRWD . . . D10.G4, DAX; TH1: AE7.A, Dorris, D1.1). Clones were either unstimulated (-) or stimulated (+) for 6 hours with plate-bound anti-CD3 ***antibody***. The positions of RNA markers, in kilobases, are shown for reference. The arrow marks the position of 200 gene mRNA.

DRWD . . . (D1.1, Dorris, AE7) cell lines and TH2 (D10.G4, DAX, CDC25) cell lines, either stimulated (+) or unstimulated (-) with anti-CD3 ***antibodies***. 15 micrograms of total RNA were loaded per lane. Cells were stimulated between 6 and 7 hours with anti-CD3 ***antibodies***, as described, below, in Section 8.1. The Northern blots were hybridized with a probe made from the entire band 54. . .

DRWD . . . (DAX, CDC25) was isolated from either unstimulated cells or from cells which had been stimulated for two hours with anti-CD3 ***antibodies***. 15 micrograms total RNA were loaded per lane. A band 54 DNA probe was used for hybridization.

DRWD . . . the full length human 200 gene. Bottom line: human 200 gene nucleotide sequence (SEQ ID NO: 23); top line: human ***200*** ***gene*** ***product*** derived amino acid sequence (SEQ ID NO:24).

DRWD . . . surface 103 gene product. The further to the right the peaks are shifted, the greater the staining intensity, and therefore ***antibody*** binding, exhibited by a cell population.

DRWD . . . 103/Ig fusion results in significant decrease in hallmark symptoms of asthma. FIG. 29A: Animals were treated with the anti-103 3E10 ***antibody*** (listed in the figure as "3E10 MAB"). As a negative control, a set of animals was treated with a non-specific rat Ig ***antibody*** preparation. FIG. 29B: Animals were treated with 103/Ig fusion protein (listed in the figure as "Ig Fus. Prot.") as a negative control, a control set of animals were treated with a non-specific human IgG ***antibody*** preparation.

DRWD . . . a section of untreated mouse kidney tissue; FIG. 31B shows a section of mouse kidney tissue treated with 200 gene ***antibody*** 24 hours prior to, and at 24 hour intervals after the induction of ***ischemic*** kidney injury.

DRWD [0113] FIG. 32. Histological scoring of gene 200 blockage in treated (+a200) and untreated (+RtIg) mouse kidney tissue during renal ***ischemia*** /reperfusion injury (RI), and in non ***ischemic*** controls (S).

DETD . . . rejection and graft versus host disease, are described. The methods and compositions described herein can also be used to treat ***ischemic*** disorders and injuries, including but not limited to, ***ischemic*** renal disease and injury, myocardial ***ischemia*** such as angina pectoris, as well as ***ischemic*** injury to other tissues, including the brain (as in a stroke), spleen, intestine, lung, and testes. Further, the methods and compositions described herein can also be used to regulate ***ischemic*** injury to other types of tissue, such as tumor tissue including, but not limited to tumors of the ovary and. . . gene products of the invention are also involved in processes related to tissue repair and remodeling after injury, particularly after ***ischemic*** injury. Thus, the genes and gene products of the invention can also be used to successfully treat such injuries and. . .

DETD [0116] Further, the gene products of such fingerprint, target, and pathway genes are described in Section 5.5, ***antibodies*** to such gene products are described in Section 5.6, as are cell- and animal-based models of TH cell subpopulation differentiation. . .

DETD . . . (b) the differentiation and effector function of TH cell subpopulations, and (c) processes related to tissue repair and remodeling after ***ischemic*** injury are described in Section 5.8. Methods for the treatment of immune disorders and ***ischemic*** disorders and injuries are described in Section 5.10.

DETD . . . limited to, pharmacological methods, such as exposure to phorbol esters, calcium ionophores, or lectins (e.g., Concanavalin A), by treatment with ***antibodies*** directed against T-cell receptor epitopes (e.g., anti-CD3 ***antibodies***) or exposure, in the presence of an appropriate antigen presenting cell (APC), to an antigen that the particular TH cells. . .

DETD . . . maintenance, and/or effector function of the subpopulations, using this protein in a manner similar to the well known technique of ***antibody*** probing of .lambda.gt11 libraries.

DETD . . . the pathway and/or differentially expressed genes of the invention also include gene products which are recognized by and bind to ***antibodies*** (polyclonal or monoclonal) directed against the differentially expressed and/or pathway gene products described above; e.g., which are encoded by the. . .

DETD . . . and/or pathway gene product including, but not limited to a human and murine 10, 54, 57, 105, 106, 161 and ***200*** ***gene*** ***product*** ; (b) nucleotides that encode portions of differentially expressed and/or pathway gene product that corresponds to its functional domains, and the. . .

DETD . . . mutant allele. In this manner, gene products made by the putatively mutant tissue can be expressed and screened using standard ***antibody*** screening techniques in conjunction with ***antibodies*** raised against the normal gene product, as described, below, in Section 5.6. (For screening techniques, see, for example,

Harlow, E. and Lane, eds., 1988, " ***Antibodies*** : A Laboratory Manual", Cold Spring Harbor Press, Cold Spring Harbor.) In cases where the mutation results in an expressed gene product with altered function (e.g., as a result of a missense mutation), a polyclonal set of ***antibodies*** are likely to cross-react with the mutant gene product. Library clones detected via their reaction with such labeled ***antibodies*** can be purified and subjected to sequence analysis as described in this Section, above.

DETD . . . as many contiguous amino acid residues as necessary to represent an epitope fragment (that is to be recognized by an ***antibody*** directed to the differentially expressed or pathway gene product). For example, such protein fragments or peptides can comprise at least. . .

DETD . . . being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of ***antibodies*** or to screen peptide libraries, for example, vectors which direct the expression of high levels of fusion protein products that. . .

DETD [0229] Alternatively, any fusion protein may be readily purified by utilizing an ***antibody*** specific for the fusion protein being expressed. For example, a system described by Janknecht et al. allows for the ready. . .

DETD [0231] Indirect labeling involves the use of a protein, such as a labeled ***antibody***, which specifically binds to either a differentially expressed or pathway gene product. Such ***antibodies*** include but are not limited to polyclonal, monoclonal, chimeric, single chain, Fab fragments and fragments produced by an Fab expression. . .

DETD [0234] Among the soluble Ig-tailed fusion proteins which can be produced are soluble Ig-tailed fusion proteins containing 103 gene products, ***200*** ***gene*** ***products*** or 10 gene products. The 103 gene product or 200 gene contained within such fusion proteins can comprise, respectively, for. . .

DETD [0239] The signal sequence, extracellular, transmembrane and cytoplasmic domains of both the murine and human ***200*** ***gene*** ***products*** have been elucidated and can be utilized in, for example, the construction of ***200*** ***gene*** ***product*** -Ig fusion proteins. Specifically, the 280 amino acid murine ***200*** ***gene*** ***product*** (FIG. 17; SEQ ID NO:10) contains a signal sequence from approximately amino acid residue 1 to approximately amino acid residue. . . a cytoplasmic domain from approximately amino acid residue 215 to amino acid residue 280. Further, the 301 amino acid human ***200*** ***gene*** ***product*** (FIG. 24; SEQ. ID. NO: 24) contains a signal sequence from amino acid residue 1 to approximately 20, a mature. . . Given the elucidation of these domains, one of skill in the art would readily be capable of producing soluble Ig-tailed ***200*** ***gene*** ***product*** fusion proteins. The Example presented, below, in Section 10 describes the construction of murine and human ***200*** ***gene*** ***product*** -Ig fusion proteins.

DETD 5.6. ***Antibodies*** Specific for Differentially Expressed or Pathway Gene Products

DETD [0241] Described herein are methods for the production of ***antibodies*** capable of specifically recognizing one or more differentially expressed or pathway gene product epitopes. Such ***antibodies*** can include, but are not limited to, polyclonal

antibodies , monoclonal ***antibodies*** (mAbs), humanized or chimeric ***antibodies*** , single chain ***antibodies*** , Fab fragments, F(ab').sub.2 fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) ***antibodies*** , and epitope-binding fragments of any of the above. The Ig tails of such ***antibodies*** can be modified to reduce complement activation and Fc binding. (See, for example, European Patent No. 239400 B1, Aug. 3, . . .

DETD [0242] Such ***antibodies*** can be used, for example, in the detection of a fingerprint, target, or pathway gene product. in a biological sample, and can be used as part of diagnostic techniques. Alternatively, such ***antibodies*** can be utilized as part of an immune disorder treatment method, as described, below, in Section 5.9. For example, the ***antibodies*** can be used to modulate target gene activity, can be used to modulate TH cell subpopulation differentiation, maintenance and/or effector function, or, in the case of ***antibodies*** directed to cell surface epitopes, can be used to isolate a TH cell subpopulation of interest, for either depletion or.

DETD [0243] Such ***antibodies*** can also be utilized as part of a method for treatment of an ***ischemic*** disorder or injury, as described in Section 5.10.3.1, below. For example, the ***antibodies*** can be used to block or inhibit activity of one or more of the gene products of the invention, thereby reducing or inhibiting repair of certain ***ischemic*** tissues, for example carcinogenic tumors.

DETD [0244] For the production of ***antibodies*** to a differentially expressed or pathway gene, various host animals can be immunized by injection with a differentially expressed or. . .

DETD [0245] Polyclonal ***antibodies*** are heterogeneous populations of ***antibody*** molecules derived from the sera of animals immunized with an antigen, such as target gene product, or an antigenic functional derivative thereof. For the production of polyclonal ***antibodies*** , host animals such as those described above, can be immunized by injection with differentially expressed or pathway gene product supplemented. . .

DETD [0246] Monoclonal ***antibodies*** , which are homogeneous populations of ***antibodies*** to a particular antigen, can be obtained by any technique which provides for the production of ***antibody*** molecules by continuous cell lines in culture. These include, but are not limited to the hybridoma technique of Kohler and. . . 4:72; Cole et al., 1983, Proc. Natl. Acad. Sci. USA 80:2026-2030), and the EBV-hybridoma technique (Cole et al., 1985, Monoclonal ***Antibodies*** And Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). Such ***antibodies*** can be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD and any subclass thereof. The hybridoma producing the. . .

DETD [0247] In addition, techniques developed for the production of "chimeric ***antibodies*** " (Morrison et al., 1984, Proc. Natl. Acad. Sci., 81:6851-6855; Neuberger et al., 1984, Nature, 312:604-608; Takeda et al., 1985, Nature, 314:452-454; U.S. Pat. No. 4,816,567) by splicing the genes from a mouse ***antibody*** molecule of appropriate antigen specificity together with genes from a human ***antibody*** molecule of appropriate biological activity can be used. A chimeric ***antibody*** is a molecule in which different portions are derived

from different animal species, such as those having a variable region.

DET D [0248] Alternatively, techniques described for the production of single chain ***antibodies*** (U.S. Pat. No. 4,946,778; Bird, 1988, Science 242:423-426; Huston et al., 1988, Proc. Natl. Acad. Sci. USA 85:5879-5883; and Ward et al., 1989, Nature 334:544-546) and for making humanized monoclonal ***antibodies*** (U.S. Pat. No. 5,225,539, which is incorporated herein by reference in its entirety) can be utilized to produce anti-differentially expressed or anti-pathway gene product ***antibodies***.

DET D [0249] ***Antibody*** fragments which recognize specific epitopes can be generated by known techniques. For example, such fragments include but are not limited to: the F(ab').sub.2 fragments which can be produced by pepsin digestion of the ***antibody*** molecule and the Fab fragments which can be generated by reducing the disulfide bridges of the F(ab').sub.2 fragments. Alternatively, Fab. . .

DET D [0250] ***Antibodies*** to the differentially expressed or pathway gene products can, in turn, be utilized to generate anti-idiotypic ***antibodies*** that "mimic" such gene products, using techniques well known to those skilled in the art. (See, e.g., Greenspan & Bona, . . J 7(5):437-444; and Nissinoff, 1991, J. Immunol. 147(8):2429-2438). For example, in the case of receptor-type molecules (e.g., 10, 103 and ***200*** ***gene*** ***products***) ***antibodies*** which bind to the ECD and competitively inhibit the binding of ligand to the receptor can be used to generate. . .

DET D [0251] Production of ***antibodies*** directed against the extracellular domain of the 103 gene product are described in Section 12, below. Also, production of ***antibodies*** directed against the extracellular domain of the ***200*** ***gene*** ***product*** are described in Section 13, below.

DET D . . . from the animal, in situ hybridization analysis, and RT-PCR. Samples of target gene-expressing tissue, can also be evaluated immunocytochemically using ***antibodies*** specific for the target gene transgene gene product of interest.

DET D [0265] The target gene transgenic animals that express target gene mRNA or target gene transgene peptide (detected immunocytochemically, using ***antibodies*** directed against target gene product epitopes) at easily detectable levels can then be further evaluated to identify those animals which. . .

DET D . . . the interaction of the target gene product with other cellular proteins. For example, in the cases of 10, 103 and ***200*** ***gene*** ***products***, which are or are predicted to be transmembrane receptor-type proteins, such techniques can identify ligands for such receptors. A compound. . . the basis for amelioration of such TH2-like-specific disorders as asthma or allergy, given that gene 103 expression is TH2-specific. A ***200*** ***gene*** ***product*** ligand can, for example, act as the basis for amelioration of TH1-like-specific disorders. A 10 gene product ligand can, for. . .

DET D . . . limited to members of random or partially degenerate, directed phosphopeptide libraries; see, e.g., Songyang, Z. et al., 1993, Cell 72:767-778), ***antibodies*** (including, but not limited to polyclonal, monoclonal, humanized, anti-idiotypic, chimeric or single chain ***antibodies***, and FAb, F(ab').sub.2 and FAb expression library fragments, and epitope-binding fragments thereof), and small

organic or inorganic molecules. In the . . . bind to the ECD and either mimic the activity triggered by the natural ligand (i.e., agonists); as well as peptides, ***antibodies*** or fragments thereof, and other organic compounds that mimic the ECD (or a portion thereof) and bind to a "neutralize". . .

DETD . . . can be accomplished by simply coating the solid surface with a solution of the protein and drying. Alternatively, an immobilized ***antibody***, preferably a monoclonal ***antibody***, specific for the protein to be immobilized can be used to anchor the protein to the solid surface. The surfaces. . .

DETD . . . is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; e.g., using a labeled ***antibody*** specific for the previously nonimmobilized component (the ***antibody***, in turn, can be directly labeled or indirectly labeled with a labeled anti-Ig ***antibody***).

DETD . . . be conducted in a liquid phase, the reaction products separated from unreacted components, and complexes detected; e.g., using an immobilized ***antibody*** specific for target gene product or the test compound to anchor any complexes formed in solution, and a labeled ***antibody*** specific for the other component of the possible complex to detect anchored complexes.

DETD . . . target gene protein, especially mutant target gene proteins. Such compounds can include, but are not limited to molecules such as ***antibodies***, peptides, and the like, as described, for example, in Section 5.8.1. above.

DETD . . . coating the solid surface with a solution of the target gene product or binding partner and drying. Alternatively, an immobilized ***antibody*** specific for the species to be anchored can be used to anchor the species to the solid surface. The surfaces. . .

DETD . . . is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; e.g., using a labeled ***antibody*** specific for the initially non-immobilized species (the ***antibody***, in turn, can be directly labeled or indirectly labeled with a labeled anti-Ig ***antibody***). Depending upon the order of addition of reaction components, test compounds which inhibit complex formation or which disrupt preformed complexes. . .

DETD . . . or absence of the test compound, the reaction products separated from unreacted components, and complexes detected; e.g., using an immobilized ***antibody*** specific for one of the binding components to anchor any complexes formed in solution, and a labeled ***antibody*** specific for the other partner to detect anchored complexes. Again, depending upon the order of addition of reactants to the. . .

DETD . . . the resulting fusion protein. The interactive cellular or extracellular binding partner can be purified and used to raise a monoclonal ***antibody***, using methods routinely practiced in the art and described above, in Section 5.6. This ***antibody*** can be labeled with the radioactive isotope ¹²⁵I, for example, by methods routinely practiced in the art. In a heterogeneous. . . binding to occur. At the end of the reaction period, unbound material can be washed away, and the labeled monoclonal ***antibody*** can be added to the system and allowed to bind to the complexed components. The interaction between the target gene. . .

DETD . . . away. Again the extent of inhibition of the target gene product/binding partner interaction can be detected by adding the

labeled ***antibody*** and measuring the radioactivity associated with the beads.

DETD . . . describes the successful utilization of a 103 gene product/Ig fusion protein, as well as the successful use of a monoclonal ***antibody*** directed against the extracellular domain of the 103 gene product to ameliorate symptoms of asthma in an accepted animal model. . .

DETD . . . limited to members of random or partially degenerate, directed phosphopeptide libraries; see, e.g., Songyang, Z. et al., 1993, Cell 72:767-778), ***antibodies*** (including, but not limited to polyclonal, monoclonal, humanized, anti-idiotypic, chimeric or single chain ***antibodies***, and FAb, F(ab').sub.2 and FAb expression library fragments, and epitope-binding fragments thereof), and small organic or inorganic molecules.

DETD . . . soluble Ig-tailed fusion proteins which may be produced by utilizing techniques such as those described, above, in Section 5.5. Additionally, ***antibodies*** directed against the extracellular portion of the 10 gene product may reduce 10 gene product function by, for example, blocking. . .

DETD . . . herein can also be used for treating, ameliorating, or modulating symptoms associated with mast cell-related processes or disorders or certain ***ischemia*** disorders or injuries. For example, techniques which increase the expression or activity of certain gene products of the invention whose activity is involved in the repair of ***ischemic*** injury or damage, particularly techniques which increase the expression or activity of the ***200*** ***gene*** ***product*** of this invention, can be used to treat tissue or organ damage produced by an ***ischemic*** disorder or injury. Alternatively, techniques or methods which inhibit the expression or activity of target gene products of the invention can be used to block or inhibit the repair of ***ischemic*** tissue or organs. Such techniques are useful, for example, to treat ***ischemic*** or infarcted tissue, such as a cancerous tumor, to increase damage or injury to such tissue, e.g., during treatment such. . .

DETD [0341] Among the ***ischemic*** disorders or injuries whose symptoms can be ameliorated are ***ischemic*** renal disease and myocardial ***ischemia***, such as angina pectoris, as well as ***ischemic*** injuries to other tissues including, but by no means limited to, the brain (as in a stroke), spleen, intestine, lung, and testes. Such techniques can also be used to treat, or to enhance ***ischemic*** injuries to tumors, including tumors of the ovary or uterus.

DETD [0342] The methods described herein can additionally be used to treat or prevent ***ischemic*** damage or injury to transplanted organs, such as transplanted kidneys, lungs, hearts, livers, and pancreas, or grafts, such as skin grafts. Such techniques are important in that some ***ischemic*** damage to transplanted organs typically occurs during transplant from donor to host, when oxygen perfusion to tissue of the transplanted. . .

DETD . . . is demonstrated in Section 13, herein, to play a critical role in the resolution (i.e., the repair) of injury following ***ischemia*** reperfusion. The 200 gene and its products can, therefore, be utilized in the treatment of ***ischemic*** disorders and injuries. For example, a gene 200 product, or functional portions thereof, can be utilized either directly or indirectly to stimulate or increase the repair of injury to tissue or organs resulting from an

ischemic injury or disorder.

DETD . . . above, techniques which serve to inhibit the expression or activity of target gene products of the present invention, including the ***200*** ***gene*** ***product***, can be used to inhibit the repair or recovery of certain ***ischemic*** tissue.

DETD . . . with the invention to ameliorate certain TH cell subpopulation-related disorder symptoms, or, alternatively, to inhibit the repair or recovery of ***ischemic*** tissue. As discussed in Section 5.8, above, such molecules can include, but are not limited to peptides (such as, for example, peptides representing soluble extracellular portions of target gene product transmembrane receptors), phosphopeptides, small organic or inorganic molecules, or ***antibodies*** (including, for example, polyclonal, monoclonal, humanized, anti-idiotypic, chimeric or single chain ***antibodies***, and FAb, F(ab').sub.2 and FAb expression library fragments, and epitope-binding fragments thereof). In one embodiment, for example, ***antibodies*** directed against a 103 gene product, preferably an extracellular or extracellular portion of a 103 gene product, can be utilized.. . .

DETD . . . IgE immune responses, including, but not limited to the symptoms which accompany atopic conditions such as allergy and/or asthma. IgE-type ***antibodies*** are produced by stimulated B cells which require, at least in part, IL-4 produced by the TH2 cell subpopulation. Therefore,. . .

DETD [0351] For example, natural ligands, derivatives of natural ligands and ***antibodies*** which bind to the 103 gene product can be utilized to reduce the number of TH2 cells present by either. . .

DETD . . . TH1 cell subpopulation involved in the disorder of interest. As such, a number of methods exist whereby the TH1 specific ***200*** ***gene*** ***products*** can be used to effect such a reduction in the activity and/or effective concentration of TH1 cells.

DETD [0357] For example, natural ligands, derivatives of natural ligands and ***antibodies*** which bind to the ***200*** ***gene*** ***product*** can be utilized to reduce the number of TH1 cells present by either physically separating such cells away from other. .

DETD [0358] Additionally, compounds can be administered which compete with endogenous ligand for the ***200*** ***gene*** ***product***. Such compounds would bind to and "neutralize" circulating ligand. The resulting reduction in the amount of ligand-bound 200 gene transmembrane. . . Further, reduction in the amount of ligand bound 200 gene transmembrane protein will also inhibit the resolution and repair of ***ischemic*** tissue.

DETD . . . and/or analogs thereof, of the gene 200 product, including, for example, soluble fusion proteins such as Ig-tailed fusion proteins or ***antibodies***. (For a discussion of the production of Ig-tailed fusion proteins see, for example, U.S. Pat. No. 5,116,964.)

DETD [0360] To this end, peptides corresponding to the ECD of the ***200*** ***gene*** ***product***, soluble deletion mutants of ***200*** ***gene*** ***product***, or either of these ***200*** ***gene*** ***product*** domains or mutants fused to another polypeptide (e.g., an IgFc polypeptide) can be utilized. Alternatively, anti-idiotypic ***antibodies*** or Fab fragments of antiidiotypic ***antibodies*** that mimic the ***200*** ***gene*** ***product*** ECD and neutralize ***200*** ***gene***

product ligand can be used. Such ***200*** ***gene***
product peptides, proteins, fusion proteins, anti-idiotypic
antibodies or Fabs are administered to a subject in amounts
sufficient to neutralize the gene product and thereby effectuate an
amelioration of a T cell subpopulation-related disorder, or an
inhibition of repair of ***ischemic*** tissues.

DETD [0361] ***200*** ***gene*** ***product*** peptides
corresponding to the ECD having the amino acid sequence shown in FIG. 17
(SEQ ID NO:10) from about amino acid residue number 21 to about 192 can
be used. Human ***200*** ***gene*** ***product*** peptides
corresponding to the ECD having the amino acid sequence shown in FIG. 24
(SEQ ID NO:24) from approximately amino. . .

DETD [0362] In an alternative embodiment for neutralizing circulating
200 ***gene*** ***product*** ligand, cells that are
genetically engineered to express such soluble or secreted forms of
200 ***gene*** ***product*** may be administered to a
patient, whereupon they will serve as "bioreactors" in vivo to provide a
continuous supply of the ***200*** ***gene*** ***product***
ligand neutralizing protein. Such cells may be obtained from the patient
or an MHC compatible donor and can include, but. . . cells etc. The
cells are genetically engineered in vitro using recombinant DNA
techniques to introduce the coding sequence for the ***200***
gene ***product*** peptide, or ***200*** ***gene***
product fusion proteins (discussed above) into the cells, e.g.,
by transduction (using viral vectors, and preferably vectors that
integrate the transgene. . . cell genome) or transfection procedures,
including but not limited to the use of plasmids, cosmids, YACs,
electroporation, liposomes, etc. The ***200*** ***gene***
product coding sequence can be placed under the control of a
strong constitutive or inducible promoter or promoter/enhancer to
achieve expression and secretion of the 200 gene peptide or fusion
protein. The engineered cells which express and secrete the desired
200 ***gene*** ***product*** can be introduced into the
patient systemically, e.g., in the circulation, or intraperitoneally.
Alternatively, the cells can be incorporated into. . .

DETD [0364] It is to be understood that, while such approaches and techniques
are described, for sake of clarity, using the ***200*** ***gene***
product as an example, they may be applied to any of the target
and/or pathway gene products having such receptor-type structures.

DETD . . . into the tissue site, or modified antisense molecules, designed
to target the desired cells (e.g., antisense linked to peptides or
antibodies that specifically bind receptors or antigens
expressed on the target cell surface) can be administered systemically.

DETD . . . gene expression or to increase the activity of target gene
product can serve to enhance the repair or resolution of
ischemia reperfusion injury to an organ or tissue. Thus, such
techniques can also be used to successfully treat ***ischemic***
disorders or injuries.

DETD . . . activity can be used in accordance with the invention to
ameliorate certain TH cell subpopulation-related disorder symptoms, or
to treat ***ischemic*** disorders and injuries. As discussed in
Section 5.8, above, such molecules can include, but are not limited to
proteins or. . . also include peptides representing soluble
extracellular portions of target gene product transmembrane proteins,
phosphopeptides, small organic or inorganic molecules, or

antibodies (including, for example, polyclonal, monoclonal, humanized, anti-idiotypic, chimeric or single chain ***antibodies***, and FAb, F(ab.sub.1).sub.2 and FAb expression library fragments, and epitope-binding fragments thereof).

DETD . . . symptoms, be administered to a patient exhibiting such symptoms. Such compounds can also be administered to a patient having an ***ischemic*** disorder or injury at a level sufficient to ameliorate the symptoms of the ***ischemic*** disorder or injury. Any of the techniques discussed, below, in Section 5.11, can be utilized for such administration. One of. . .

DETD . . . target gene product in vivo. For example, in one preferred embodiment, fragments or peptides representing a functional domain of the ***200*** ***gene*** ***product*** are administered to a patient which enhance and/or mimic the activity of the endogenous ***200*** ***gene*** ***product*** in that patient. Such 200 gene fragments may therefore be used, e.g., to stimulate or increase the repair of injury to tissue or organs resulting from an ***ischemic*** injury or disorder.

DETD . . . results for the elicitation of a reduction in the immune disorder symptoms, or in a reduction of the symptoms of ***ischemic*** disorders and injuries. In the case of compounds which act intracellularly, the DNA molecules encoding such peptides must be taken.

DETD [0395] In cases wherein such molecules are administered to treat an ***ischemia*** related disorder or injury, preferred techniques are those which serve to selectively administer DNA molecules to the affected organ or tissue. For example, in the case of ***ischemic*** renal disease, techniques for the selective administration of the molecules to cells within the kidney are preferred.

DETD . . . a sufficient circulating concentration of the extracellular molecule for the amelioration of immune disorders, or to ameliorate the symptoms of ***ischemic*** disorders or injuries. Further, as above, when the gene encodes a cell which acts intracellularly or as a transmembrane molecule,. . .

DETD . . . which are sufficient to ameliorate the symptoms of T cell subpopulation related disorders, or, alternatively, to ameliorate the symptoms of ***ischemic*** disorders or injuries. Such cells may be either recombinant or non-recombinant. Among the cells which can be administered to increase. . .

DETD . . . including TH cell subpopulation-related disorders. Further, the modulatory techniques described in Section 5.10.3.1, below, can also be used to treat ***ischemic*** disorders and injuries, or, alternatively, to block or inhibit repair of ***ischemic*** injuries depending on whether such modulation is positive or negative. Thus, in appropriate instances, the procedures of this Section can. . .

DETD 5.10.3.1. ***Antibody*** Techniques

DETD [0402] ***Antibodies*** exhibiting modulatory capability can be utilized to ameliorate immune disorders such as TH cell subpopulation-related disorders, or to treat ***ischemic*** disorders and injuries. Depending on the specific ***antibody***, the modulatory effect can be negative and can, therefore, be utilized as part of the techniques described, above, in Section. . .

DETD [0403] An ***antibody*** having negative modulatory capability refers to an ***antibody*** which specifically binds to and interferes with the action of a protein. In the case of an extracellular

receptor, for example, such an ***antibody*** would specifically bind the extracellular domain of the receptor in a manner which does not activate the receptor but which disrupts the ability of the receptor to bind its natural ligand. For example, ***antibodies*** directed against the extracellular domains of genes 103 or 200 can function as such negative modulators. Additionally, ***antibodies*** directed against one or more of the 10 gene product extracellular domains can function in a negative modulatory manner. Such ***antibodies*** can be generated using standard techniques described in Section 5.6, above, against full length wild type or mutant proteins, or against peptides corresponding to portions of the proteins. The ***antibodies*** include but are not limited to polyclonal, monoclonal, FAb fragments, single chain ***antibodies***, chimeric ***antibodies***, and the like.

DETD [0404] An ***antibody*** having positive modulatory capability refers to an ***antibody*** which specifically binds to a protein and, by binding, serves to, either directly or indirectly, activate the function of the protein which it recognizes. For example, an ***antibody*** can bind to the extracellular portion of a transmembrane protein in a manner which causes the transmembrane protein to function as though its endogenous ligand was binding, thus activating, for example, a signal transduction pathway. ***antibodies*** can be generated using standard techniques described in Section 5.6, above, against full length wild type or mutant proteins, or against peptides corresponding to portions of the proteins. The ***antibodies*** include but are not limited to polyclonal, monoclonal, FAb fragments, single chain ***antibodies***, chimeric ***antibodies***, and the like.

DETD [0405] In instances where the protein, such as a target gene protein, to which the ***antibody*** is directed is intracellular and whole ***antibodies*** are used, internalizing ***antibodies*** can be preferred. However, lipofectin or liposomes can be used to deliver the ***antibody*** or a fragment of the Fab region which binds to the gene product epitope into cells. Where fragments of the ***antibody*** are used, the smallest inhibitory fragment which binds to the protein's binding domain is preferred. For example, peptides having an amino acid sequence corresponding to the domain of the variable region of the ***antibody*** that binds to the protein can be used. Such peptides can be synthesized chemically or produced via recombinant DNA technology. . . . methods well known in the art (e.g., see Creighton, 1983, supra; and Sambrook et al., 1989, above). Alternatively, single chain ***antibodies***, such as neutralizing ***antibodies***, which bind to intracellular epitopes can also be administered. Such single chain ***antibodies*** can be administered, for example, by expressing nucleotide sequences encoding single-chain ***antibodies*** within the target cell population by utilizing, for example, techniques such as those described in Marasco et al. (Marasco, W. . . .

DETD [0406] In instances where the protein to which the ***antibody*** is directed is extracellular, or is a transmembrane protein, any of the administration techniques described, below in Section 5.11 which are appropriate for peptide administration can be utilized to effectively administer the ***antibodies*** to their site of action.

DETD . . . markers. Such markers can include, but are not limited to, the TH2-specific 103 gene product extracellular domain markers, the TH1-specific ***200*** ***gene*** ***product***

extracellular domain markers and the TH inducible 10 gene product extracellular domain markers.

DETD . . . of the separation is to increase or augment the number of cells belonging to a specific TH cell subpopulation, the ***antibodies*** used can also be specific to surface markers present on undifferentiated or partially undifferentiated TH cells. After separation, and purification. . .

DETD . . . such as TH1 or TH2 cells, can be removed, and thus selectively separated and purified, by various methods which utilize ***antibodies*** which bind specific markers present on the T cell population of interest, while absent on other cells within the starting. . . a fluorescence activated cell sorter (FACS) and specific fluorochromes, biotin-avidin or biotin-streptavidin separations using biotin conjugated to cell surface marker-specific ***antibodies*** and avidin or streptavidin bound to a solid support such as affinity column matrix or plastic surfaces or magnetic separations using ***antibody*** -coated magnetic beads.

DETD [0413] Separation via ***antibodies*** for specific markers can be by negative or positive selection procedures. In negative separation, ***antibodies*** are used which are specific for markers present on undesired cells. For example, in the case of a TH1 cell subpopulation-related disorder wherein it would be desirable to deplete the number of TH1 cells, such ***antibodies*** could be directed to the extracellular domain of the ***200*** ***gene*** ***product***. Alternatively, in the case of TH2 cell subpopulation-related disorders wherein it would be desirable to deplete the number of TH1 cells, such ***antibodies*** could be directed to the extracellular domain of the 103 gene product. Cells bound by an ***antibody*** to such a cell surface marker can be removed or lysed and the remaining desired mixture retained.

DETD [0414] In positive separation, ***antibodies*** specific for markers present on the desired cells of interest. For example, in the case of a TH1 cell subpopulation-related disorder wherein it would be desirable to increase the number of TH1 cells, such ***antibodies*** could be directed to the extracellular domain of the ***200*** ***gene*** ***product***. Alternatively, in the case of TH2 cell subpopulation-related disorders wherein it would be desirable to increase the number of TH1 cells, such ***antibodies*** could be directed to the extracellular domain of the 103 gene product. Cells bound by the ***antibody*** are separated and retained. It will be understood that positive and negative separations can be used substantially simultaneously or in. . .

DETD [0415] A common technique for ***antibody*** based separation is the use of flow cytometry such as by a fluorescence activated cell sorter (FACS). Typically, separation by flow cytometry is performed as follows. The suspended mixture of cells are centrifuged and resuspended in media. ***Antibodies*** which are conjugated to fluorochrome are added to allow the binding of the ***antibodies*** to specific cell surface markers. The cell mixture is then washed by one or more centrifugation and resuspension steps. The. . .

DETD . . . such method is biotin-avidin based separation by affinity chromatography. Typically, such a technique is performed by incubating cells with biotin-coupled ***antibodies*** to specific markers, such as, for example, the transmembrane protein encoded by the 103 gene described herein, followed by passage through an avidin column. Biotin-

antibody -cell complexes bind to the column via the biotin-avidin interaction, while other cells pass through the column. The specificity of the. . .

DETD . . . proliferation of the purified TH cell subpopulation. Such an expansion agent can, for example, be any appropriate cytokine, antigen, or ***antibody***. In the case of TH2 cells, for example, the expansion agent can be IL-4, while for TH1 cells, the expansion. . .

DETD [0425] Targeting moieties can include, but are not limited to, ***antibodies*** directed to cell surface markers found specifically on the TH cell subpopulation being targeted, or, alternatively, to ligands, such as. . .

DETD [0426] In the case of TH2 cells, for example, such a targeting moiety can represent an ***antibody*** directed against the extracellular portion of the 103 gene product described herein, or can, alternatively, represent a ligand specific for. . . this receptor-type TH2 specific molecule. In the case of TH1 cells, for example, such a targeting moiety can represent an ***antibody*** directed against the extracellular portion of the ***200*** ***gene*** ***product*** described herein, or can, alternatively, represent a ligand specific for this receptor-type TH1 specific molecule.

DETD [0431] As defined herein, a therapeutically effective amount of ***antibody***, protein, or polypeptide (i.e., an effective dose or effective dosage) ranges from about 0.001 to 30 mg/kg of body weight,. . .

DETD . . . subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a protein, polypeptide or ***antibody*** can include a single treatment or, preferably, can include a series of treatments. In a preferred example, a subject is treated with ***antibody***, protein, or polypeptide in the range of between about 0.1 to 20 mg/kg body weight one time per week for. . . even more preferably for about 4, 5, or 6 weeks. It will also be appreciated that the effective dosage of ***antibody***, protein or polypeptide used for treatment may increase or decrease over the course of a particular treatment. Changes in dosage. . .

DETD 5.11.3. Pharmaceutical Preparation and Administration of ***Antibodies***

DETD [0444] ***Antibodies*** which specifically bind to target gene products of the invention and thereby modulate their activity can also be administered to a patient at therapeutically effective doses to treat or ameliorate immune disorders, or to treat or ameliorate ***ischemic*** disorders or injuries. For example, Section 13, below, demonstrates the use of anti- ***200*** ***gene*** ***product*** ***antibodies*** to block recovery of kidney tissue from ***ischemia*** reperfusion injury.

DETD [0445] ***Antibodies*** of the invention are administered by any suitable means, including those described in Section 5.12.2, above. In addition, ***antibody*** to a target gene product of the invention is suitably administered by pulse infusion, particularly with declining doses of the ***antibody***. Preferably, the dosing is administered by injections, most preferably by intravenous or subcutaneous injections, depending in part on whether the. . .

DETD [0446] The appropriate dosage of ***antibody*** will depend on the type of disease to be treated, the severity and course of the disease, whether the ***antibody*** is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history

and response to the ***antibody***, and the discretion of the attending physician. The ***antibody*** may suitably administered to the patient at one time or, more preferably, over a series of treatments.

DETD [0447] As a general proposition, the initial pharmaceutically effective amount of ***antibody*** administered parenterally will be in the range of about 0.1 to 20 mg/kg of patient body weight per day, with.

DETD [0453] Such methods can, for example, utilize reagents such as the fingerprint gene nucleotide sequences described in Sections 5.1, and ***antibodies*** directed against differentially expressed and pathway gene peptides, as described, above, in Sections 5.5 (peptides) and 5.6 (***antibodies***). Specifically, such reagents can be used, for example, for: 1) the detection of the presence of target gene expression, target.

DETD . . . performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one specific fingerprint gene nucleic acid or anti-fingerprint gene ***antibody*** reagent described herein, which can be conveniently used, e.g., in clinical settings, to diagnose patients exhibiting TH1- or TH2-related abnormalities.

DETD . . . stimulating the cells prior to contacting the cells with the compound. Among the methods for stimulation are stimulation via anti-CD3 ***antibody*** stimulation.

DETD [0478] ***Antibodies*** directed against wild type or mutant fingerprint gene peptides, which are discussed, above, in Section 5.6, can also be used.

DETD . . . employed herein can, for example, be such as those described in Harlow and Lane (Harlow, E. and Lane, D., 1988, " ***Antibodies*** : A Laboratory Manual", Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.), which is incorporated herein by reference in its.

DETD . . . molecules can involve, for example, immunoassays wherein fingerprint gene peptides are detected by their interaction with an anti-fingerprint gene product-specific ***antibody***.

DETD [0481] For example, ***antibodies***, or fragments of ***antibodies***, such as those described, above, in Section 5.6, useful in the present invention can be used to quantitatively or qualitatively. . . wild type or mutant fingerprint gene peptides. This can be accomplished, for example, by immunofluorescence techniques employing a fluorescently labeled ***antibody*** (see below, this Section,) coupled with light microscopic, flow cytometric, or fluorimetric detection. Such techniques are especially preferred if the. . . peptides are expressed on the cell surface, such as, for example, is the case with the 10 gene product, the ***200*** ***gene*** ***product*** and the transmembrane form of 103 gene product. Thus, the techniques described herein can be used to detect specific cells.

DETD [0482] The ***antibodies*** (or fragments thereof) useful in the present invention can, additionally, be employed histologically, as in immunofluorescence or immunoelectron microscopy, for. . . peptides. In situ detection can be accomplished by removing a histological specimen from a patient, and applying thereto a labeled ***antibody*** of the present invention. The ***antibody*** (or fragment) is preferably applied by overlaying the labeled ***antibody*** (or fragment) onto a biological sample. Through the use of such a procedure, it is possible to determine not only.

DETD . . . extract, freshly harvested cells, or cells which have been incubated in tissue culture, in the presence of a detectably labeled ***antibody*** capable of identifying fingerprint gene peptides, and detecting the bound ***antibody*** by any of a number of techniques well-known in the art.

DETD . . . soluble proteins. The support can then be washed with suitable buffers followed by treatment with the detectably labeled fingerprint gene-specific ***antibody***. The solid phase support can then be washed with the buffer a second time to remove unbound ***antibody***. The amount of bound label on solid support can then be detected by conventional means.

DETD [0485] By "solid phase support or carrier" is intended any support capable of binding an antigen or an ***antibody***. Well-known supports or carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, gabbros, and magnetite.. . . have virtually any possible structural configuration so long as the coupled molecule is capable of binding to an antigen or ***antibody***. Thus, the support configuration can be spherical, as in a bead, or cylindrical, as in the inside surface of a . . . strip, etc. Preferred supports include polystyrene beads. Those skilled in the art will know many other suitable carriers for binding ***antibody*** or antigen, or will be able to ascertain the same by use of routine experimentation.

DETD [0486] The binding activity of a given lot of anti-wild type or mutant fingerprint gene product ***antibody*** can be determined according to well known methods. Those skilled in the art will be able to determine operative and. . .

DETD [0487] One of the ways in which the fingerprint gene peptide-specific ***antibody*** can be detectably labeled is by linking the same to an enzyme and use in an enzyme immunoassay (EIA) (Voller,. . . Raton, Fla.; Ishikawa, E. et al., (eds.), 1981, ENZYME IMMUNOASSAY, Kaku Shoin, Tokyo). The enzyme which is bound to the ***antibody*** will react with an appropriate substrate, preferably a chromogenic substrate, in such a manner as to produce a chemical moiety. . . be detected, for example, by spectrophotometric, fluorimetric or by visual means. Enzymes which can be used to detectably label the ***antibody*** include, but are not limited to, malate dehydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, yeast alcohol dehydrogenase, alpha-glycerophosphate, dehydrogenase, triose phosphate isomerase,. . .

DETD [0488] Detection can also be accomplished using any of a variety of other immunoassays. For example, by radioactively labeling the ***antibodies*** or ***antibody*** fragments, it is possible to detect fingerprint gene wild type or mutant peptides through the use of a radioimmunoassay (RIA). . .

DETD [0489] It is also possible to label the ***antibody*** with a fluorescent compound. When the fluorescently labeled ***antibody*** is exposed to light of the proper wavelength, its presence can then be detected due to fluorescence. Among the most. . .

DETD [0490] The ***antibody*** can also be detectably labeled using fluorescence emitting metals such as .sup.152Eu, or others of the lanthanide series. These metals can be attached to the ***antibody*** using such metal chelating groups as diethylenetriaminepentaacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA).

DETD [0491] The ***antibody*** also can be detectably labeled by coupling

it to a chemiluminescent compound. The presence of the chemiluminescent-tagged ***antibody*** is then determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of. . .

DETD [0492] Likewise, a bioluminescent compound can be used to label the ***antibody*** of the present invention. Bioluminescence is a type of chemiluminescence found in biological systems in, which a catalytic protein increases. . .

DETD . . . samples were isolated from either unstimulated cells or from cells which had been stimulated for 6 hours with plate-bound anti-CD3 ***antibody***. The samples were probed with band 103 sequences, as shown in FIG. 6. While 103 gene RNA is present in. . .

DETD . . . E. et al., John Wiley & Sons, NY, pp 3.12.4-3.12.6). Specifically, prior to plating, the flasks were coated with anti-CD3-.epsilon. ***antibodies*** (hybridoma supernatant from the 145-C11 hybridoma; Parmingen, Inc., San Diego Calif.). For coating, ***antibodies*** were resuspended in PBS at 1-2 .mu.g/ml at a volume sufficient to coat the bottom of the flasks. Coating solution. . .

DETD [0551] After incubation, the ***antibody*** coating solution was removed by aspiration and cells were immediately added. Flasks were placed in a 37.degree. C. incubator for. . .

DETD . . . ID NO:8) of the murine 200 gene coding region, as shown in FIG. 17. FIG. 17 also depicts the murine ***200*** ***gene*** ***product*** derived amino acid sequence (SEQ ID NO:10). Database searches reveal that the ***200*** ***gene*** ***product*** is a novel receptor which contains an extracellular Ig domain, thus placing it within the Ig receptor superfamily. The cloning. . .

DETD . . . samples were isolated from either unstimulated cells (-) or cells which had been stimulated for 6 hours with plate-bound anti-CD3 ***antibody*** (+). The samples were probed with 200 gene sequences, and, as shown in FIG. 18, RNA from both stimulated and. . .

DETD . . . blot data depicted in FIG. 19 additionally illustrates 54 gene expression within cell clones either stimulated or unstimulated with anti-CD3 ***antibodies***, and demonstrates that 54 gene expression goes down within stimulated TH1 cells.

DETD . . . Specifically, RNA was isolated from unstimulated AE7 clones as well as from AE7 clones which had been stimulated with anti-CD3 ***antibodies*** for varying lengths of time, as noted in FIG. 20. As illustrated in FIG. 20, 54 gene expression decreased slightly. . .

DETD . . . open reading frame is depicted in FIG. 24 (SEQ ID NO: 37). The derived amino acid sequence of the human ***200*** ***gene*** ***product*** is also depicted in FIG. 24 (SEQ ID NO: 24).

DETD [0593] The 301 amino acid residue sequence of the human ***200*** ***gene*** ***product*** reveals that it is a cell surface receptor exhibiting distinct domains, including a signal sequence from amino acid residue 1. . . Ig type variable set domain from approximately amino acid residue 30 to approximately amino acid residue 128, thus placing the ***200*** ***gene*** ***product*** within the Ig receptor superfamily.

DETD . . . 200 gene, has been successfully cloned and characterized, as described herein. As revealed by its amino acid sequence, the human ***200*** ***gene*** ***product*** is a receptor of the Ig superfamily class of molecules.

DETD . . . the murine 200 gene-hIgG1 fusion protein vector, the CD5 and CD44 portions of pCD5-CD44-IgG1 were replaced with sequences encoding

murine ***200*** ***gene*** ***product*** signal sequence and extracellular domain.

DETD [0617] The construction and expression of recombinant IgG fusion proteins is described herein. Specifically, ***200*** ***gene*** ***product*** -IgG1 and 103 gene product-IgG1 fusion proteins are described. The murine and human ***200*** ***gene*** ***product*** -IgG1 fusion protein contains a ***200*** ***gene*** ***product*** signal sequence and extracellular domain fusion to a human IgG1 heavy chain Fc region. The 103 gene product-IgG1 fusion protein. . .

DETD [0619] Described herein is the production and characterization of transgenic mice overexpressing either murine ***200*** ***gene*** ***product*** or murine 103 gene product.

DETD . . . herein presents in vivo data demonstrating that the 103 gene product regulates TH2 effector cell responses. In particular, a monoclonal ***antibody*** (3E10 mAb) has been generated against the 103 gene product, and its effect in an adoptive transfer model of TH1.

DETD [0646] Rat monoclonal ***antibodies*** (MAbs), including the 3E10 MAb, were generated against the extracellular domain of the mouse 103 gene product. A DNA sequence. . .

DETD . . . culture supernatant (or 1 .mu.g purified 3E10 protein) was applied 1.times.10.sup.6 cells. After rinsing, cells were contacted with goat anti-rat ***antibody*** conjugated with PE (R-phycoerythrin) fluorescent dye. After a final rinse, cell analysis was carried out on a FACS Vantage (Becton. . .

DETD [0682] To further investigate the expression and role of the 103 gene product in TH cells, a monoclonal ***antibody*** (3E10 mAb) directed against the extracellular domain of the 103 gene product was prepared and characterized.

DETD . . . corroborating the results presented in the Example of Section 7, above. In addition, the data demonstrate the feasibility of using ***antibodies*** to separate TH2 subpopulation cells away from other cell types, thereby modulating a TH cell subpopulation by changing the number. . .

DETD The ***200*** ***Gene*** ***Product*** Exhibits a Critical Role in the Resolution of Injury Following Kidney ***Ischemia*** and Reperfusion

DETD [0700] The Example provided herein presents in vivo data that the ***200*** ***gene*** ***product*** is involved in the recovery from kidney ***ischemia*** injury. In particular, a monoclonal ***antibody*** (96.3.8H7 mAb) generated against the extracellular domain of the murine ***200*** ***gene*** ***product***, and its effect in a surgical model of recovery from kidney ***ischemia*** injury was investigated. The anti-200 mAb markedly impaired restoration of renal function, as determined by several indicators.

DETD [0701] These results, therefore, provide in vivo animal data demonstrating that the ***200*** ***gene*** ***product*** provides a critical function in the resolution of injury following kidney ***ischemia***. Thus, the 200 gene and its gene product can be used to aid in the recovery from ***ischemic*** injuries such as stroke, heart attack, acute renal failure, and organ transplant.

DETD [0703] Rat monoclonal ***antibodies*** (MAbs) were generated against the extracellular domain of the mouse ***200*** ***gene*** ***product***. Recombinant murine 200 gene IgG1 (m200Ig) fusion

protein was produced and purified as described in Section 10.1 above. Briefly, a DNA sequence containing the extracellular domain of the mouse ***200*** ***gene*** ***product*** was PCR-amplified and cloned into a vector containing the CD5 signal sequence and the human IgG1 constant region. COS cells. . .

DETD . . . animal were fused with SP2/0 myeloma cells, and resulting clones were screened and selected to be specific for the murine ***200*** ***gene*** ***product*** on the basis of their reactivity to clonal TH2 cell lines (DAX, and D10.G4) and thymocytes from m200 transgenic mice. . .

DETD [0707] For ***200*** ***gene*** ***product*** blockage experiments, mice were pretreated 24 hours before surgery with 100 .mu.g/mouse of rat anti-200 monoclonal ***antibody*** (200 mAb). In control experiments, mice were administered equivalent dosages of rat Ig (RtIg) ***antibody*** .

DETD [0708] Mice were then given ***antibody*** at 24 hour intervals following surgical recovery (4 hours post anesthetic).

DETD [0712] The ***200*** ***gene*** ***product*** is similar to the rat adhesion molecule called KIM-1 (Ichimura et al., 1998, J. Biol. Chem. 273:4135), levels of which are increased in cells after ***ischemic*** /reperfusion injury. The role of the ***200*** ***gene*** ***product*** is ***ischemic*** injury was investigated in vivo using a mouse model of acute renal failure. In particular, this animal model was used to investigate whether neutralization of the ***200*** ***gene*** ***product*** in vivo affected tissue repair after an ***ischemic*** injury.

DETD [0713] A monoclonal ***antibody*** (96.3.8H7 mAb) directed against the extracellular domain of the ***200*** ***gene*** ***product*** was prepared and characterized, and this ***antibody*** was administered to mice 24 hours before, and at 24 hour intervals following ***ischemic*** kidney injury. Control animals were administered equal dosages of rat Ig at the same intervals. A second group of animals underwent sham surgery, described in Section 13.1 above, wherein the animals were not subjected to ***ischemic*** kidney injury.

DETD . . . 3 below. Creatinine and blood urea nitrogen levels returned to basal levels in untreated (+RtIg) mice within 72 hours post ***ischemia*** . However, mice treated with anti-200 mAb (+a200) maintained elevated levels of both blood urea nitrogen (122.5 mg/dl vs. 34.3 mg/dl). . .

DETD [0716] In summary, these results provide in vivo animal data demonstrating that the ***200*** ***gene*** ***product*** plays a critical role during recovery from ***ischemic*** kidney injury, and can be used as a novel treatment to aid in the recovery from ***ischemic*** injuries such as acute renal failure.

DETD . . . high levels of 103 gene expression in a human mast cell line. The example also describes the production of monoclonal ***antibodies*** which are specific for the human, but not mouse, 103 gene product. FAC staining of the human mast cell line demonstrated binding of these monoclonal ***antibodies*** , confirming that the 103 gene product is, indeed, expressed in mast cells.

DETD [0719] Monoclonal ***antibodies*** (mAbs; see below) were generated against the extracellular domain (amino acid residues 18-323) of a human 103 gene product (with. . .

DETD . . . protein using fluorescence activated cells sorting (FACS)

according to standard methods described in Section 12.1, above using anti-mouse IgFITC secondary ***antibodies***.

DETD . . . in a human mast cell line. Expression of the 103 gene product in this cell line was verified using monoclonal ***antibodies*** raised against an Fc fusion protein of the human 103 gene product, as described in Section 14.1, above.

DETD [0732] FACS staining of the human mast cell line, with the 21 monoclonal ***antibodies*** showed staining with 15 of the 21 ***antibodies*** compared to isotype controls. Five of these 15 ***antibodies***, identified as 1B4, 2O3, 3F7, 3H18, and 10F7, were selected for further analysis. FAC staining with these ***antibodies*** was demonstrated to be specifically blocked with an excess of human 103-Fc fusion protein, however, staining was not blocked with. . .

DETD . . . is expressed in a human mast cell line. Accordingly, the 103 gene, its gene product, and compositions derived therefrom (e.g., ***antibodies*** and other compounds which bind to and/or modulate the expression or activity of the 103 gene or its gene product). . . include, but are not limited to, atherosclerosis (see, e.g., Metzler and Xu, 1997, Int. Arch. Allergy Immunol. 114:10-14) and myocardial ***ischemia*** /reperfusion (see, e.g., Frangogiannis et al., 1998, Circulation 98:699-710).

CLM What is claimed is:

1. A method for ameliorating a symptom of an ***ischemic*** disorder or injury in a mammal, comprising administering to the mammal a ***200*** ***gene*** ***product*** in an amount effective to ameliorate the symptom of the ***ischemic*** disorder or injury.

2. A method for ameliorating a symptom of an ***ischemic*** disorder or injury in a mammal, comprising administering to the mammal a nucleic acid molecule encoding a ***200*** ***gene*** ***product*** in an amount effective to ameliorate the symptom of the ***ischemic*** disorder or injury.

3. A method for ameliorating a symptom of an ***ischemic*** disorder or injury in a mammal, comprising administering to the mammal an ***antibody*** directed against a ***200*** ***gene*** ***product*** in an amount effective to ameliorate the symptom of the disorder.

4. The method of claim 1, 2, or 3, wherein the ***ischemic*** disorder is ***ischemic*** renal disease, or myocardial ***ischemia***.

5. The method of claim 4, wherein the myocardial ***ischemia*** is angina pectoris.

6. The method of claim 1, 2, or 3 wherein the ***ischemic*** disorder or injury is a infarction.

8. The method of claim 1, 2, or 3, wherein the ***ischemic*** injury is to a transplanted organ.

10. The method of claim 1, 2, or 3, wherein the ***200*** ***gene*** ***product*** is a polypeptide comprising: (a) the amino acid sequence of SEQ ID NO:10, (b) the amino acid sequence

encoded by. . .

11. The method of claim 1, 2, or 3, wherein the ***200***
gene ***product*** is a polypeptide encoded by a nucleic
acid molecule which hybridizes under highly stringent conditions to the
complement of: (a). . .

13. The method of claim 1 wherein said administering of the ***200***
gene ***product*** is parenteral, subcutaneous,
intraperitoneal, intrapulmonary, intranasal, or intralesional.

. . . 14. The method of claim 13, wherein the intralesional administration
comprises perfusing or contacting a graft or organ with the ***200***
gene ***product*** before transplant.

17. The method of claim 3 wherein said administering of the
antibody is parenteral, subcutaneous, intraperitoneal,
intrapulmonary, intranasal, or intralesional.

. . . 18. The method of claim 17, wherein the intralesional administration
comprises perfusing or contacting a graft or organ with the
antibody before transplant.

19. The method of claim 3, wherein the amount of the ***antibody***
administered is from about 1 .mu.g/kg to about 100 mg/kg.

20. The method of claim 19, wherein the amount of the ***antibody***
administered is from about 1 .mu.g/kg to about 15 mg/kg.

21. The method of claim 20, wherein the amount of the ***antibody***
administered is from about 0.1 mg/kg to about 2.0 mg/kg.

L2 ANSWER 3 OF 7 USPATFULL on STN

AN 2003:3427 USPATFULL

TI Novel human interleukin-like proteins and polynucleotides encoding them

IN Fernandes, Elma, Branford, CT, UNITED STATES

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PI US 2003003462 A1 20030102

AI US 2001-965212 A1 20010926 (9)

RLI Continuation of Ser. No. US 2000-544511, filed on 6 Apr 2000, ABANDONED

PRAI US 2000-186592P 20000303 (60)

US 1999-128514P 19990409 (60)

DT Utility

FS APPLICATION

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CLMN Number of Claims: 71

ECL Exemplary Claim: 1

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CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention provides novel isolated SECX polynucleotides and
the membrane-associated or secreted polypeptides encoded by the SECX
polynucleotides. Also provided are the ***antibodies*** that

immunospecifically bind to a SECX polypeptide or any derivative, variant, mutant or fragment of the SECX polypeptide, polynucleotide or ***antibody***. The invention additionally provides methods in which the SECX polypeptide, polynucleotide and ***antibody*** are utilized in the detection and treatment of a broad range of pathological states, as well as to other uses.

AB . . . provides novel isolated SECX polynucleotides and the membrane-associated or secreted polypeptides encoded by the SECX polynucleotides. Also provided are the ***antibodies*** that immunospecifically bind to a SECX polypeptide or any derivative, variant, mutant or fragment of the SECX polypeptide, polynucleotide or ***antibody***. The invention additionally provides methods in which the SECX polypeptide, polynucleotide and ***antibody*** are utilized in the detection and treatment of a broad range of pathological states, as well as to other uses.

SUMM [0002] The invention relates to polynucleotides and polypeptides encoded by such polynucleotides, as well as vectors, host cells, ***antibodies*** and recombinant methods for producing the polypeptides and polynucleotides.

SUMM [0011] Also included in the invention is an ***antibody*** which selectively binds to a SECX polypeptide.

SUMM . . . nucleic acid in a sample from a mammal, e.g., a human, by contacting a sample from the mammal with an ***antibody*** which selectively binds to one of the herein described polypeptides, and detecting the formation of reaction complexes including the ***antibody*** and the polypeptide in the sample. Detecting the formation of complexes in the sample indicates the presence of the polypeptide in the sample. Methods for measurements of ***antibody*** reaction complex concentrations are well known in the art. Methods for detecting and quantitating nucleic acids include hybridization and TaqMan.TM.. . .

SUMM [0018] Alternatively, the mammal may be treated by administering an ***antibody*** as herein described in an amount sufficient to alleviate the pathological condition.

DETD . . . having 180 residues (WO9932632-A1, published JUL. 1, 1999). The EDIRF-like DNA and protein sequences (e.g., Clone 2191999) and their homologues, ***antibodies*** (Ab) specific for EDIRF-like protein, and other modulators may be used: (i) in screening and detection assays, e.g. for chromosome. . .

DETD . . . to a fragment of human limbic system associated membrane protein (LAMP; PCT Publication WO9630052-A1, published 03-OCT-1996). LAMP is a self-binding, ***antibody*** -like cell surface adhesion protein involved in formation of connections between adjacent neurons. LAMP protein, and by analogy the clone 4324229. . .

DETD . . . Ig superfamily class. Expression of 200 gene is many-fold higher in TH1 than in TH2 subpopulations (WO9627603-A1). Modulation of the ***200*** ***gene*** ***product*** may ameliorate a range of T-cell-related disorders. BLASTP searches also show a moderate degree of similarity to kidney injury molecule-1. . .

DETD . . . (ACC:P55083 and U.S. Pat. No. 5,972,654-A, issued OCT. 25, 1999). The human microfibril-associated glycoprotein 4 splice variant (MAG4V) polypeptides and/or ***antibodies*** thereto are disclosed in this patent as being usable to down regulate MAG4V expression and activity. By analogy, Clone 4437909.0.4. . .

DETD . . . receptors or antigens expressed on a selected cell surface,

e.g., by linking the antisense nucleic acid molecules to peptides or ***antibodies*** that bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using.

DETD . . . or derivatives, fragments, analogs or homologs thereof. Also provided are polypeptide fragments suitable for use as immunogens to raise anti-SECX ***antibodies***. In one embodiment, native SECX proteins can be isolated from cells or tissue sources by an appropriate purification scheme using. . .

DETD . . . or inhibiting) cell survival. Moreover, the SECX-immunoglobulin fusion proteins of the invention can be used as immunogens to produce anti-SECX ***antibodies*** in a subject, to purify SECX ligands, and in screening assays to identify molecules that inhibit the interaction of SECX. . .

DETD [0199] Anti-SECX ***Antibodies***

DETD [0200] The invention encompasses ***antibodies*** and ***antibody*** fragments, such as F.sub.ab or (F.sub.ab).sub.2, that bind immunospecifically to any of the polypeptides of the invention.

DETD [0201] An isolated SECX protein, or a portion or fragment thereof, can be used as an immunogen to generate ***antibodies*** that bind SECX using standard techniques for polyclonal and monoclonal ***antibody*** preparation. The full-length SECX protein can be used or, alternatively, the invention provides antigenic peptide fragments of SECX for use. . . 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30 and encompasses an epitope of SECX such that an ***antibody*** raised against the peptide forms a specific immune complex with SECX. Preferably, the antigenic peptide comprises at least 6, 8, . . .

DETD . . . which regions of a SECX polypeptide are particularly hydrophilic and, therefore, are likely to encode surface residues useful for targeting ***antibody*** production. As a means for targeting ***antibody*** production, hydropathy plots showing regions of hydrophilicity and hydrophobicity may be generated by any method well known in the art, . . .

DETD . . . 26, 28 and 30, or derivatives, fragments, analogs or homologs thereof, may be utilized as immunogens in the generation of ***antibodies*** that immunospecifically-bind these protein components. The term " ***antibody*** " as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that specifically binds (immunoreacts with) an antigen, such as SECX. Such ***antibodies*** include, but are not limited to, polyclonal, monoclonal, chimeric, single chain, F.sub.ab and F.sub.(ab')₂ fragments, and an F.sub.ab expression library. In a specific embodiment, ***antibodies*** to human SECX proteins are disclosed. Various procedures known within the art may be used for the production of polyclonal or monoclonal ***antibodies*** to a SECX protein sequence, or derivative, fragment, analog or homolog thereof. Some of these proteins are discussed below.

DETD [0204] For the production of polyclonal ***antibodies***, various suitable host animals (e.g. rabbit, goat, mouse or other mammal) may be immunized by injection with the native protein, . . . oil emulsions, dinitrophenol, etc.), human adjuvants such as Bacille Calmette--Guerin and Corynebacterium parvum, or similar immunostimulatory agents. If desired, the ***antibody*** molecules directed against SECX can be isolated from the mammal (e.g., from the blood) and further purified by

well known. . .

DETD [0205] The term "monoclonal ***antibody*** " or "monoclonal ***antibody*** composition", as used herein, refers to a population of ***antibody*** molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of SECX. A monoclonal ***antibody*** composition thus typically displays a single binding affinity for a particular SECX protein with which it immunoreacts. For preparation of monoclonal ***antibodies*** directed towards a particular SECX protein, or derivatives, fragments, analogs or homologs thereof, any technique that provides for the production of ***antibody*** molecules by continuous cell line culture may be utilized. Such techniques include, but are not limited to, the hybridoma technique. . . hybridoma technique (see Kozbor, et al., 1983 Immunol Today 4: 72) and the EBV hybridoma technique to produce human monoclonal ***antibodies*** (see Cole, et al., 1985 In: MONOCLONAL ***ANTIBODIES*** AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96). Human monoclonal ***antibodies*** may be utilized in the practice of the present invention and may be produced by using human hybridomas (see Cote, . . . 80: 2026-2030) or by transforming human B-cells with Epstein Barr Virus in vitro (see Cole, et al., 1985 In: MONOCLONAL ***ANTIBODIES*** AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96). Each of the above citations are incorporated herein by reference in. . .

DETD [0206] According to the invention, techniques can be adapted for the production of single-chain ***antibodies*** specific to a SECX protein (see e.g., U.S. Pat. No. 4,946,778). In addition, methodologies can be adapted for the construction. . . of monoclonal F.sub.ab fragments with the desired specificity for a SECX protein or derivatives, fragments, analogs or homologs thereof. Non-human ***antibodies*** can be "humanized" by techniques well known in the art. See e.g. U.S. Pat. No. 5,225,539. ***Antibody*** fragments that contain the idiotypes to a SECX protein may be produced by techniques known in the art including, but not limited to: (i) an F.sub.(ab')₂ fragment produced by pepsin digestion of an ***antibody*** molecule; (ii) an F.sub.ab fragment generated by reducing the disulfide bridges of an F.sub.(ab')₂ fragment; (iii) an F.sub.ab fragment generated by the treatment of the ***antibody*** molecule with papain and a reducing agent and (iv) F.sub.v fragments.

DETD [0207] Additionally, recombinant anti-SECX ***antibodies***, such as chimeric and humanized monoclonal ***antibodies***, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the invention. Such chimeric and humanized monoclonal ***antibodies*** can be produced by recombinant DNA techniques known in the art, for example using methods described in International Application No. . . .

DETD [0208] In one embodiment, methodologies for the screening of ***antibodies*** that possess the desired specificity include, but are not limited to, enzyme-linked immunosorbent assay (ELISA) and other immunologically-mediated techniques known within the art. In a specific embodiment, selection of ***antibodies*** that are specific to a particular domain of a SECX protein is facilitated by generation of hybridomas that bind to the fragment of a SECX protein possessing such a domain. ***Antibodies*** that are specific for an above-described domain within a SECX protein, or derivatives, fragments, analogs or homologs thereof, are also. . .

DETD [0209] Anti-SECX ***antibodies*** may be used in methods known within the art relating to the localization and/or quantitation of a SECX protein (e.g., . . . physiological samples, for use in diagnostic methods, for use in imaging the protein, and the like). In a given embodiment, ***antibodies*** for SECX proteins, or derivatives, fragments, analogs or homologs thereof, that contain the ***antibody*** derived binding domain, are utilized as pharmacologically-active compounds [hereinafter "Therapeutics"].

DETD [0210] An anti-SECX ***antibody*** (e.g., monoclonal ***antibody***) can be used to isolate SECX by standard techniques, such as affinity chromatography or immunoprecipitation. An anti-SECX ***antibody*** can facilitate the purification of natural SECX from cells and of recombinantly produced SECX expressed in host cells. Moreover, an anti-SECX ***antibody*** can be used to detect SECX protein (e.g., in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the SECX protein. Anti-SECX ***antibodies*** can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, e.g., to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (i.e., physically linking) the ***antibody*** to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and. . .

DETD [0237] The SECX nucleic acid molecules, SECX proteins, and anti-SECX ***antibodies*** (also referred to herein as "active compounds") of the invention, and derivatives, fragments, analogs and homologs thereof, can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the nucleic acid molecule, protein, or ***antibody*** and a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion. . .

DETD [0240] Sterile injectable solutions can be prepared by incorporating the active compound (e.g., a SECX protein or anti-SECX ***antibody***) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed. .

DETD . . . be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal ***antibodies*** to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to. . .

DETD [0250] The nucleic acid molecules, proteins, protein homologues, and ***antibodies*** described herein that include extracellular and transmembrane domains and, therefore, can be used in one or more of the following. . .

DETD . . . disorders such as cancer or preclampsia, or any disease or disorder described in Sections 1-14 above). In addition, the anti-SECX ***antibodies*** of the invention can be used to detect and isolate SECX proteins and modulate SECX activity.

DETD . . . (e.g., biotinylation kit, Pierce Chemicals, Rockford, Ill.), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, ***antibodies*** reactive with SECX or target molecules, but which do not interfere with binding of the SECX protein to its target. . . molecule, can be derivatized to the wells of the plate, and unbound target or SECX trapped in the wells by

antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using ***antibodies*** reactive with the SECX or target molecule, as well as enzyme-linked assays that rely on detecting an enzymatic activity associated. . .

DETD . . . been implicated in cardiovascular disorders, including in atherosclerotic plaque formation. Diseases such as cardiovascular disease, including cerebral thrombosis or hemorrhage, ***ischemic*** heart or renal disease, peripheral vascular disease, or thrombosis of other major vessel, and other diseases, including diabetes mellitus, hypertension,. . .

DETD . . . with a monomeric form of a peptide having an activity of another B lymphocyte antigen (e.g., B7-1, B7-3) or blocking ***antibody***), prior to transplantation can lead to the binding of the molecule to the natural ligand(s) on the immune cells without. . .

DETD . . . of inappropriate activation of T cells that are reactive against self tissue and which promote the production of cytokines and auto- ***antibodies*** involved in the pathology of the diseases. Preventing the activation of autoreactive T cells may reduce or eliminate disease symptoms.. . . by disrupting receptor:ligand interactions of B lymphocyte antigens can be used to inhibit T cell activation and prevent production of auto- ***antibodies*** or T cell-derived cytokines which may be involved in the disease process. Additionally, blocking reagents may induce antigen-specific tolerance of. . .

DETD [0338] Assays for T-cell-dependent immunoglobulin responses and isotype switching (which will identify, among others, proteins that modulate T-cell dependent ***antibody*** responses and that affect Th1/Th2 profiles) include, without limitation, those described in: Maliszewski, J Immunol 144:3028-3033, 1990; and Mond and. . .

DETD . . . acute conditions), including without limitation inflammation associated with infection (such as septic shock, sepsis or systemic inflammatory response syndrome (SIRS)), ***ischemia*** -reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine-induced lung injury, inflammatory bowel disease, Crohn's disease or resulting. . .

DETD . . . Methods include, e.g., Northern or Southern blot hybridization (above) or quantitative PCR (below) for measuring SECX nucleic acid levels, and ***antibody*** detection (below), such as ELISA assays and ***antibody*** pull-down assay for measuring SECX polypeptide levels.

DETD [0387] An agent for detecting SECX protein is an ***antibody*** capable of binding to SECX protein, preferably an ***antibody*** with a detectable label. ***Antibodies*** can be polyclonal, or more preferably, monoclonal. An intact ***antibody***, or a fragment thereof (e.g., Fab or F(ab').sub.2) can be used. The term "labeled", with regard to the probe or ***antibody***, is intended to encompass direct labeling of the probe or ***antibody*** by coupling (ie., physically linking) a detectable substance to the probe or ***antibody***, as well as indirect labeling of the probe or ***antibody*** by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary ***antibody*** using a fluorescently labeled secondary ***antibody*** and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin. The term. .

. include Southern hybridizations. Furthermore, in vivo techniques for detection of SECX protein include introducing into a subject a labeled anti-SECX ***antibody***. For example, the ***antibody*** can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging. . .

DETD . . . described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or ***antibody*** reagent described herein, which may be conveniently used, e.g., in clinical settings to diagnose patients exhibiting symptoms or family history. . .

DETD . . . be utilized include, but are not limited to, (i) an aforementioned peptide, or analogs, derivatives, fragments or homologs thereof; (ii) ***antibodies*** to an aforementioned peptide; (iii) nucleic acids encoding an aforementioned peptide; (iv) administration of antisense nucleic acid and nucleic acids. . . Science 244: 1288-1292); or (v) modulators (i.e., inhibitors, agonists and antagonists, including additional peptide mimetic of the invention or ***antibodies*** specific to a peptide of the invention) that alter the interaction between an aforementioned peptide and its binding partner.

DETD . . . inhibits one or more SECX protein activity. Examples of such inhibitory agents include antisense SECX nucleic acid molecules and anti-SECX ***antibodies***. These modulatory methods can be performed in vitro (e.g., by culturing the cell with the agent) or, alternatively, in vivo. . .

DETD . . . supernatant were harvested 72 hours after transfection and examined for h11753149 expression by Western blotting (reducing conditions) with an anti-V5 ***antibody***. FIG. 15 shows that h11753149 is expressed as a protein secreted by 293 cells that is broadly distributed around 64. . .

DETD . . . supernatant were harvested 72 hours after transfection and examined for h4437909 expression by Western blotting (reducing conditions) with an anti-V5 ***antibody***. FIG. 16 shows that h4437909 is expressed in 293 cells as three discrete secreted protein bands of 16, 40, and. . .

DETD . . . according to the manufacturer's instructions. After induction, total cells were harvested, and proteins were analyzed by Western blotting using anti-HisGly ***antibody*** (Invitrogen, Carlsbad, Calif.). FIG. 17 shows h4437909 was expressed as a 34 kDa protein in E. coli cells. This corresponds. . .

CLM What is claimed is:

21. An ***antibody*** that binds immunospecifically to the polypeptide of claim 1.

22. The ***antibody*** of claim 21, wherein said ***antibody*** is a monoclonal ***antibody***.

23. The ***antibody*** of claim 21, wherein the ***antibody*** is a humanized ***antibody***.

24. An ***antibody*** that binds immunospecifically to the polypeptide of claim 5.

25. The ***antibody*** of claim 24, wherein said ***antibody*** is a monoclonal ***antibody***.

26. The ***antibody*** of claim 24, wherein the ***antibody*** is a humanized ***antibody*** .

27. A method for determining the presence or amount of the polypeptide of claim 1 in a sample, the method comprising: (a) providing the sample; (b) contacting the sample with an ***antibody*** that binds immunospecifically to a polypeptide of claim 1; and (c) determining the presence or amount of ***antibody*** bound to said polypeptide, thereby determining the presence or amount of polypeptide in said sample.

. . . polypeptide of claim 5 in a sample, the method comprising: (a) providing the sample; (b) contacting the sample with an ***antibody*** that binds immunospecifically to a polypeptide of claim 5; and (c) determining the presence or amount of ***antibody*** bound to said polypeptide, thereby determining the presence or amount of polypeptide in said sample.

. . . preventing a SECX-associated disorder, said method comprising administering to a subject in which such treatment or prevention is desired the ***antibody*** of claim 21 in an amount sufficient to treat or prevent said SECX-associated disorder in said subject.

51. A pharmaceutical composition comprising the ***antibody*** of claim 21 and a pharmaceutically acceptable carrier.

52. A pharmaceutical composition comprising the ***antibody*** of claim 24 and a pharmaceutically acceptable carrier.

. . . wherein said therapeutic is selected from the group consisting of a SECX polypeptide, a SECX nucleic acid, and a SECX ***antibody*** .

70. A method of treating a pathological state in a mammal, the method comprising administering to the mammal the ***antibody*** of claim 21 in an amount sufficient to alleviate the pathological state.

71. A method of treating a pathological state in a mammal, the method comprising administering to the mammal the ***antibody*** of claim 24 in an amount sufficient to alleviate the pathological state.

L2 ANSWER 4 OF 7 USPATFULL on STN

AN 2003:81735 USPATFULL

TI Method for diagnosing and alleviating the symptoms of chronic fatigue syndrome

IN Lerner, A. Martin, 525 Harmon, Birmingham, MI, United States 48009

PI US 6537997 B1 20030325

AI US 2000-663729 20000915 (9)

RLI Continuation-in-part of Ser. No. US 1998-177942, filed on 23 Oct 1998
Continuation-in-part of Ser. No. US 802776 Division of Ser. No. US 1997-802776, filed on 18 Feb 1997

DT Utility

FS GRANTED

EXNAM Primary Examiner: Travers, Russell

LREP Brooks & Kushman P.C.

CLMN Number of Claims: 13

ECL Exemplary Claim: 1

DRWN 7 Drawing Figure(s); 2 Drawing Page(s)

LN.CNT 1638

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method for alleviating chronic fatigue syndrome with the administration of antiviral agents. Based on clinical tests, chronic fatigue syndrome is a persistent herpes virus infection including incomplete virus multiplication and thus administration of antiviral agents are shown to alleviate the symptoms associated with the disorder. Based on therapeutic trials, patients receiving the recommended antiviral treatment, have experienced significant reduction or elimination of the symptoms associated with chronic fatigue syndrome. A method of diagnosis of the chronic fatigue syndrome is further disclosed.

SUMM . . . is the basis of the CFS. The serologic evidence of EBV and HCMV is obtained by studying the level of ***antibodies*** of EBV and HCMV to detect the presence of active and persistent multiplication of either or both of the viruses. . . .

DRWD FIG. 3 is a graph depicting a 39-year old female patient's HCMV ***antibody*** titer levels following the administration of antiviral treatment; and

DRWD FIG. 4 is a graph depicting a 47-year old male patient's ***antibody*** titer levels following the administration of antiviral treatment.

DETD . . . presence of EBV and/or HCMV in CFS sufferers. Our research has further indicated for CFS sufferers the existence of IgM ***antibodies*** to the EBV viral capsid antigen (VCA) or EBV ***antibodies*** to diffuse early antigen (EA), the latter depicting EBV transactivators for the lytic cycle indicating current virus multiplication. In CFS sufferers, there may additionally or alternatively be a significant IgG immunoreactive ***antibody*** (ELISA) to HCMV, with/without an IgM (ELISA) ***antibody*** titer to HCMV.

DETD . . . that previous seroepidemiologic studies attempting to show a singular virologic causation to CFS including singular searches for EBV or HCMV ***antibodies*** would have naturally yielded uniformly negative results. At least fifteen different viruses, bacteria and parasites have been previously suspected as. . . .

DETD The herpes virus genome is capable of making over ***200*** ***gene*** ***products*** and 200 separate proteins. In FIG. 1c the latent double-stranded DNA has opened--is in its linear position and ready to. . . .

DETD . . . of viral enzymes and factors necessary for viral assembly. A complete herpes virus virion 10 is depicted in FIG. d. ***Antibodies*** to structural glycoprotein, for instance gB and gH of human cytomegalovirus, are produced. In the case of incomplete virus replication,. . . . UL44 and UL57) reach the extracellular space and are thus available to the human host immune system so that specific ***antibodies*** may be produced to these nonstructural gene products, UL44 and UL57. These nonstructural gene products are behind the virus bilayer 16 and are not exposed to ***antibody*** production when whole complete virus multiplication occurs.

DETD . . . contradictory. Just like CFS, herpes simplex virus encephalitis

(HSVE) boggled the medical community and etiologic identification due to the rising ***antibodies*** in serum which may or may not be present at a given time. Diagnosis of HSVE required isolation of herpes. . .

DETD Table 1 describes the ***antibody*** response to whole virus infection which results in ***antibodies*** to viral structural epitopes and a response to nonstructural viral epitopes such as would be seen in incomplete viral replication.. . .

DETD This Copalis.RTM. method to CM.sub.2 and p52 measures IgM ***antibodies*** to HCMV non-structural gene products. The non-structural gene products are UL44, UL57. These gene products have been produced in E-coli.. . . These are nonstructural gene products which are extruded from a cell with incomplete human cytomegalovirus multiplication. The Copalis.RTM. method measures ***antibodies*** to these two gene products with the purified antigens produced in E-coli. Table 1 shows the types of herpes virus infection with ***antibody*** responses to both structural and nonstructural viral epitopes.

DETD In virulent virus infection, the infected cell is destroyed. Cellular inflammation in the classical sense results. Specific ***antibodies*** to structural epitopes of human cytomegalovirus for instance gB and gH result. This results in IgM and IgG ***antibodies***. The IgM ***antibody***, of course, is short-lived usually less than six weeks, and the IgG ***antibody*** is long-lived. In classical virulent infection no ***antibodies*** would be produced to nonstructural viral epitopes, hereinafter referred to as p52 (to UL44 and UL57) and CM.sub.2 (UL57). In latent infection there is no complete virus produced. There is no IgM to structural epitopes gB and gH ***antibody***. IgG ***antibody***, of course, is present because there is past infection and, of course, there is no ***antibody*** to p52 or CM.sub.2. In incomplete viral multiplication, no IgM ***antibodies*** to structural epitopes are produced. Of course, IgG ***antibodies*** are stable and present. In incomplete viral multiplication, IgM ***antibodies*** are produced to nonstructural epitopes (Table 1). Finally in mixed virulent and incomplete (or complete and incomplete viral replication/multiplication), one may have IgM ***antibodies*** and IgG ***antibodies*** to structural epitopes and IgM ***antibodies*** to nonstructural epitopes.

DETD . . . one of three antigens (p52, CM.sub.2, and CMV viral particle (VP)) are dried in the reaction cup. Early phase IgM ***antibodies*** are detected by both the CM.sub.2 and the p52 antigens. ***Antibody*** response to the CM.sub.2 antigen but not to the p52 antigen continues through the convalescent stage of acute infection. ***Antibodies*** to the VP may appear during acute infection but shall persist during life. Reactivity just (sucrose-density purified tissue culture produced complete HCMV cytomegalovirus) to the VP is characteristic of prior infection (indicative of immune status). ***Antibody*** detection, made by the Copalis.RTM. Immunoassay System, is reported qualitatively for the IgM analytes and semi-quantitatively for the total analyte.

DETD The Copalis.RTM. CMVplex ***Antibody*** Assay is based on the principle of ***antibody*** dependent particle aggregation as measured by the amount of light scattered. A typical ***antibody*** reactivity pattern (IgM and/or IgG) in the patient sample allows diagnosis of the infection as seronegative, acute or past infections..

DETD Copalis.RTM. CMVplex ***Antibody*** Assay--Sized polystyrene microparticles are coated with the following antigens:

DETD The Copalis.RTM. light scattering technique thus allows for detection and precise measurement of ***antibodies*** to HCMV. It should be noted that p52 and CM.sub.2 ***antibody*** may also be detected by ELISA methods.

DETD The EA ***antibody*** is measured in the diagnosis of the chronic fatigue syndrome due to Epstein-Barr virus infection. This polymeric EA ***antibody*** to diffuse early antigen products is an ***antibody*** to as many as 12 nonstructural gene products, which are transactivators for the lytic cycle of Epstein-Barr virus so that the paradigm of complete and incomplete viral multiplication in ***antibodies*** in structural and nonstructural epitopes outlined for human cytomegalovirus relates to human Epstein-Barr virus as well. Both, of course, are. . .

DETD . . . by the therapy. One indicator of an improvement in EBV-isolated CFS patients is a decrease in the level of IgM ***antibodies*** to the VCA for EBV. Generally, the therapy duration is proportional to the intensity of the CFS manifestation. Accordingly, following. . .

DETD Serum ***antibody*** titers to Epstein-Barr virus (EBV) and cytomegalovirus (HCMV) were assayed in a consecutive case series of 98 patients with the. . . CFS patients had evidence of a cardiomyopathy documented by the presence of abnormal left ventricular dynamics at stress/rest (MUGA) tests. ***Antibodies*** to Epstein-Barr virus (EBV) immunofluorescent total early antigens (EA-D) and cytomegalovirus (HCMV) IgM and IgG enzyme immunoassays (ELISA) were measured. The prevalence of these ***antibodies*** was compared to that in a non-CFS group of control persons from the same area. Approximately 50% of CFS and non-CFS patients had concurrent EBV multiplication when tested by the presence of an elevated EA ***antibody*** titer to the (EAD) diffuse complex which neutralizes EBV encoded DNA polymerase. Elevated IgM HCMV ***antibodies*** were uncommon (<10%) in all groups. Approximately 40% of the CFS patients and non-CFS controls had no IgG HCMV ***antibody***. These data are consistent with our understanding that CFS is a persistent cardiomyopathic infection caused by EBV or HCMV, or. . .

DETD . . . serologic evidence that the human cardiac myocyte harbors EBV, HCMV or both viruses in CFS patients, we assayed EBV serum ***antibody*** total Early Antigen (EA) and IgM ***antibodies*** to the viral capsid antigen (VCA) and HCMV IgM and IgG serum ***antibodies***. However, the diagnosis of another important herpes virus infection, namely, herpes simplex virus (HSV) encephalitis was not possible by tests of specific HSV ***antibodies*** in serum. Brain biopsy with isolation of HSV, type 1 or type 2 from affected sites was necessary for diagnosis.. . .

DETD . . . radioisotopic gated blood pool method. Rest/stress myocardial perfusion studies (thallium 201 or TC-99m sestamibi) or, as necessary, cardiac catheterizations excluded ***ischemic*** cardiomyopathy in each case. CFS patient's demographic data were similar to other series. The mean age here was 42.3+-.10.6 years,. . .

DETD ***Antibody*** Titers. At the initial clinical visit of each CFS patient, EBV total early antigens (EA) immunofluorescent ***antibody*** titers (Roche Laboratories, Columbus, Ohio) and cytomegalovirus (HCMV) IgM enzyme immunoassays (ELISA) (Detroit Biomedical Laboratories, Detroit, Mich.) and HCMV ELISA. . . IgG and IgM species onto the solid phase by anti-human immunoglobulin followed by the addition of antigen and labeling the ***antibodies***. With

regard to IgM assays, this ELISA method reduces the potential interference of rheumatoid factor. During the EBV infectious lytic. . (EA-R) complex. We assayed the EA-D. The 52/50 KD.sub.a EA-D protein complex neutralizes EBV encoded DNA polymerase activity. EBV EA ***antibody*** indicates recent EBV multiplication (e.g. within 90 days). Likewise, HCMV ELISA IgM enzyme immunoassays indicate recent virus multiplication. A positive. . . IgG titer does not differentiate concurrent from past HCMV infection. As a control group to the CFS patients, similar serum ***antibody*** titers were measured in 20 random well individuals from the same area. They were 60% women with a mean age. . .

DETD The prevalence of EBV EA ***antibodies*** were similar in all CFS patients, both those with and those without abnormal left ventricular dynamics. The prevalence of these EBV ***antibodies*** were also the same in non-CFS well persons. Approximately one-half of each of the groups, CFS and non-CFS, had concurrent persistent multiplication of EBV as indicated by an EA ***antibody*** titer of .gtoreq.10. Similarly, IgG HCMV ***antibodies*** were present in approximately 60% of each group. However, HCMV, IgM ***antibodies*** were uncommon (<10%).

DETD . . . was demonstrated, were treated and studied over a six month period. EBV active infection was documented by EBV VCA IgM ***antibodies*** and/or EBV EA elevated ***antibody*** titers. All of these patients had their energy levels reduced by 50% or more and were partially or entirely non-functional,. . .

DETD During the initial and final clinical visits, each CFS patient was studied to determine the IgM ***antibodies*** to the EBV viral capsid antigen (VCA), total early antigens (EA), and enzyme immunoassays (ELISA) to HCMV IgM and HCMV ELISA IgG titers. An IgM EBV VCA ***antibody*** titer indicates infectious lytic EBV multiplication within the most recent 3 month period. During the EBV infectious lytic cycle, antigen. . . and a cytoplasmic restricted EAR complex (EAR). As a result, each patient's EAD was assayed. The presence of EBV EA ***antibody*** indicates current EBV multiplication. Likewise, an elevated cytomegalovirus or Epstein-Barr virus IgM ***antibody*** indicates recent EBV or HCMV multiplication, respectively. The EBV VCA ***antibody*** IgM titers were done by the ELISA method. As a control group, similar serum ***antibody*** titers were measured in 20 non-CFS individuals. In the non-CFS control group, the mean HCMV IgG titer was 240.

DETD . . . VCA, IgM titers in five out of ten. The remainder of the EBV suspected CFS patients had elevated EBV EA ***antibody*** titers. At the completion of the trial, two out of ten continued to have VCA IgM positive titers. At the beginning of the trial, seven out of ten had positive EA ***antibody*** titers (.gtoreq.10). At the completion of the trial, eight out of ten continued to have positive EA ***antibody*** titers. This population of ten patients was characterized by little to no HCMV experience. None had a HCMV IgM titer;. . .

DETD . . . to assess the possible efficacy of ganciclovir treatment on a subset of CFS patients with (1) high HCMV IgG ELISA ***antibody*** titers; (2) minimal/no serologic evidence of concurrent EBV multiplication; and (3) oscillating ECG abnormalities at Holter monitoring.

DETD . . . HCMV ELISA IgG titers, as well as EBV capsid antigen (VCA), ELISA IgM and EBV total early antigens (EA) immunofluorescent

antibody titers were assayed. Additionally, buffy coats, urine and myocardial biopsies were tested for infectious HCMV. As a control of the occurrence of HCMV and EBV virus ***antibodies*** in normal non-CFS persons, residing in the same area, 20 random individuals were tested for HCMV ***antibodies*** and for EBV ***antibodies***. Holter monitoring, MUGA studies and endomyocardial biopsies in these patients were done.

DETD HCMV and EBV ***Antibody*** Titers. Eight of the patients had high HCMV ELISA IgG ***antibody*** titers with a mean of 322 u. Two patients had positive IgM HCMV ***antibody*** titers. Ten of the 13 patients had little to no evidence of EBV multiplication as tested by an elevated ***antibody*** titer to EA.

DETD . . . normal. At resting 12-lead ECG showed an inverted T-wave in standard lead III, but was otherwise normal. An ELISA IgM ***antibody*** titer to HCMV was positive, while Epstein-Barr virus ***antibody*** tests were negative. Holter monitoring showed oscillating abnormal flat or inverted T-waves appearing with the onset of sinus tachycardias, and. . .

DETD A myocardial sestamibi perfusion rest/stress test showed reversible ***ischemia*** of the anterior, apical and inferior walls, but at cardiac catheterization, the coronary arteries were patent. A stress MUGA study. . .

DETD Conclusion. Based upon these research results, it is understood that CFS patients with a significant ELISA IgG HCMV ***antibody*** titer, greater than 120 units, with or without the presence of an IgM HCMV ELISA ***antibody*** titer plus an absence of EBV VCA IgM ***antibody*** titer along with an EBV EA ***antibody*** titer less than 40 describes a group of CFS patients that are understood to derive benefit from ganciclovir treatment. Our. . .

DETD . . . Patients. Results of Copalis.RTM. Assays Specificity: The serum of 17 patients were studied who had no cytomegalovirus IgG or IgM ***antibody*** to structural viral proteins as measured by the classical ELISA method. In these 17 patients there were no ***antibodies*** by the Copalis.RTM. method using light scattering technique to either viral protein (VP), CM.sub.2 or p52. Therefore, no false-positives by. . .

DETD . . . the 121 separate patients were similarly tested. These patients were positive by the ELISA classical technique for structural cytomegalovirus IgG ***antibody***. They were negative for cytomegalovirus IgM ***antibody***. All 121 of these patients positive for cytomegalovirus IgG ***antibody*** were positive for viral protein (Copalis.RTM.). The antigen for the cytomegalovirus ELISA technique is nonpurified, human fibroblast tissue culture suspension. . . cases which were positive for cytomegalovirus IgG by both Copalis.RTM. and ELISA methods, they were negative by Copalis.RTM. method for ***antibodies*** to p52 and CM.sub.2. None of these patients had the chronic fatigue syndrome. There was one patient who was positive in very low titer to cytomegalovirus IgG by the Copalis.RTM. method which was negative for IgG ***antibody*** to cytomegalovirus by the ELISA method. These data indicate that Copalis.RTM. light scatter ***antibodies*** do not occur with nonstructural proteins CM.sub.2 or p52 in patients with past cytomegalovirus infection. Seventy-six sera were analyzed which were positive for viral capsid antigen IgM to Epstein-Barr virus and/or EA ***antibody*** to Epstein-Barr virus. These cases had no cytomegalovirus ELISA IgM or IgG. The obtained serum

in 76 cases was uniformly negative by Copalis.RTM. tests to human cytomegalovirus IgG and IgM. The IgM includes both nonstructural ***antibodies*** CM.sub.2 and p52. Therefore Epstein-Barr virus did not cause false-positive Copalis.RTM. results. The tests by the Copalis.RTM. method are greater. . .

DETD Results of Copalis.RTM. ***antibody*** testing in patients with the U.S. CDC--Chronic Fatigue Syndrome and various other patients with severe onset of explained fatigue. Group. . . chronic fatigue syndrome. They are six women and eight men with ages ranging from 32 through 48. They had IgG ***antibodies*** to human cytomegalovirus. They had no IgM ***antibodies*** to human cytomegalovirus by the ELISA technique. They would be evaluated as having past infection. These eight patients had high titer positive ***antibodies*** to nonstructural gene products p52 and CM.sub.2. They were treated with intravenous ganciclovir and followed by oral ganciclovir. These nonstructural ***antibodies*** became markedly lower and disappeared. Moreover, the fatigue in these patients was remarkably lessened. The use of the Copalis.RTM. ***antibody*** titer in these patients depicted incomplete virus multiplication and the reversal of this immune status associated with the improvement of. . . a 27 year old woman and a 48 year old woman had similar evidence of chronic fatigue syndrome and high ***antibody*** titers to the nonstructural gene products of human cytomegalovirus p52 and CM.sub.2. These patients could not be treated with anti-viral agent, ganciclovir. Nevertheless, their initial diagnosis was secured by the presence of the ***antibodies*** to p52 and CM.sub.2.

DETD FIG. 4 is a table depicting the ***antibody*** titer levels of one of the patients, a 47-year old male, before and after antiviral therapy. This patient had IgG ***antibodies*** to HCMV but no IgM ***antibodies*** by the ELISA technique. As shown, this patient had high titer positive ***antibodies*** to nonstructural gene products p52 and CM.sub.2. After antiviral treatment, these nonstructural ***antibodies*** became markedly lower.

DETD . . . with the chronic fatigue syndrome are of interest. In two of these patients, the patient had, of course, IgG ELISA ***antibodies*** to human cytomegalovirus and no IgM ***antibodies*** to the ELISA testing. They would be ordinarily read as having past infection. They had, however, markedly high ***antibody*** titers to CM.sub.2. They had no elevated ***antibody*** titers to p52. They were treated with intravenous ganciclovir followed by oral ganciclovir. The CM.sub.2 ***antibodies*** lessened or disappeared and they improved. The third patient, a 39 year old woman, with chronic fatigue syndrome was diagnosed. . . This patient was similarly treated with intravenous ganciclovir followed by oral ganciclovir and improved remarkably. Therefore, the decreasing response to ***antibodies*** to HCMV nonstructural gene products (CM.sub.2) after specific antiviral administration defines human cytomegalovirus chronic fatigue syndrome and define three kinds. . . Several other patients who had the sudden onset of ongoing, unexplained fatigue also have been studied and found to have ***antibodies*** to the p52 and CM.sub.2. One of these was an 83 year old woman with congestive heart failure and diabetes. . . after intravenous ganciclovir. A second patient is a 75 year old woman with fatigue. Both of these older women had ***antibody*** responses to both CM.sub.2 and p52. A 60 year old man with morbid obesity and diabetes mellitus suddenly became fatigued. . . fatigue. He also was

identified as having incomplete cytomegalovirus infection by the presence of high titers of the two nonstructural ***antibodies***. Finally, an 82 year old woman had ongoing fatigue of unknown cause. She had ***antibodies*** only to the p52 epitopes.

DETD . . . depicted in FIG. 3, the administration of antiviral therapy, specifically both ganciclovir and valacyclovir, remarkably reduced the presence of high ***antibody*** titers to both HCMV and EBV.

DETD . . . invention provides an exact means of diagnosing a subset of patients with cytomegalovirus chronic fatigue syndrome. The decrease in these ***antibody*** titers has been identified and the use of specific anti-viral therapy has resulted in significant patient improvement, accompanied by a. . .

CLM What is claimed is:

. . . for serologic evidence of EBV and HCMV, further comprising: obtaining serum from the patient; measuring the level of EBV IgM ***antibodies*** to the VCA in the serum; measuring the level of EBV ***antibodies*** to the total EA in the serum; measuring the level of HCMV IgM ***antibodies*** in the serum by measuring antigens p52 and CM.sub.2 with the use of a light scattering technique; measuring the level of HCMV IgG ***antibodies*** in the serum by measuring antigens p52 and CM.sub.2 with the use of a light scattering technique; monitoring the patient. . . of the chronic fatigue syndrome when the measurements show any one of the following: 1) an elevated level of IgM ***antibodies*** to the VCA for EBV; and 2) presence of total EA ***antibodies*** for EBV, in combination with the absence of IgM ***antibodies*** for HCMV and a low level of IgG ***antibodies*** for HCMV; classifying HCMV as the cause of the chronic fatigue syndrome when the measurements show any one of the following: 1) an elevated level of IgM ***antibodies*** for HCMV; and 2) an elevated level of IgG ***antibodies*** for HCMV, in combination with a low level of IgM ***antibodies*** to the VCA for EBV, and the absence of total EA ***antibodies*** for EBV; classifying a combination of EBV and HCMV as the cause of the chronic fatigue syndrome when the measurements show any one of the following: 1) an elevated level of IgM ***antibodies*** to the VCA for EBV; and 2) the presence of total EA ***antibodies*** for EBV, in combination with any of the following: 1) an elevated level of IgM ***antibodies*** for HCMV; and 2) an elevated level of IgG ***antibodies*** for HCMV; administering to the patient a therapeutically effective amount of one or more pharmaceutically acceptable antiviral agents suitable for. . .

L2 ANSWER 5 OF 7 USPATFULL on STN

AN 2002:307875 USPATFULL

TI Novel KIAA1061-like cell adhesion molecule-like proteins and polynucleotides encoding them

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Vernet, Corine, Gainesville, FL, UNITED STATES

Shimkets, Richard A., West Haven, CT, UNITED STATES

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PI US 2002172999 A1 20021121

AI US 2001-966545 A1 20010926 (9)

RLI Continuation of Ser. No. US 2000-544511, filed on 6 Apr 2000, ABANDONED

PRAI US 2000-186592P 20000303 (60)

US 1999-128514P 19990409 (60)

DT Utility

FS APPLICATION

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CLMN Number of Claims: 88

ECL Exemplary Claim: 1

DRWN 42 Drawing Page(s)

LN.CNT 7282

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This application is drawn to novel amino acid sequences for mammalian polypeptides that have sequence similarity to human cell adhesion molecule KIAA1061, and processes for preparing the same. The clone identified has a nucleic acid sequence that is 4000 nucleotides long, contains an open reading frame from nucleotides 408-410 and 2934-2936, and encodes a novel polypeptide that is 842 amino acids long

SUMM [0003] The invention relates to polynucleotides and polypeptides encoded by such polynucleotides, as well as vectors, host cells, ***antibodies*** and recombinant methods for producing the polypeptides and polynucleotides.

SUMM [0012] Also included in the invention is an ***antibody*** which selectively binds to a SECX polypeptide.

SUMM . . . nucleic acid in a sample from a mammal, e.g., a human, by contacting a sample from the mammal with an ***antibody*** which selectively binds to one of the herein described polypeptides, and detecting the formation of reaction complexes including the ***antibody*** and the polypeptide in the sample. Detecting the formation of complexes in the sample indicates the presence of the polypeptide in the sample. Methods for measurements of ***antibody*** reaction complex concentrations are well known in the art. Methods for detecting and quantitating nucleic acids include hybridization and TaqMan.TM.. . .

SUMM [0019] Alternatively, the mammal may be treated by administering an ***antibody*** as herein described in an amount sufficient to alleviate the pathological condition.

DETD . . . having 180 residues (WO9932632-A1, published Jul. 01, 1999). The EDIRF-like DNA and protein sequences (e.g., Clone 2191999) and their homologues, ***antibodies*** (Ab) specific for EDIRF-like protein, and other modulators may be used: (i) in screening and detection assays, e.g. for chromosome. . .

DETD . . . fragment of human limbic system associated membrane protein (LAMP; PCT Publication WO9630052-A1, published Oct. 03, 1996). LAMP is a self-binding, ***antibody*** -like cell surface adhesion protein involved in formation of connections between adjacent neurons. LAMP protein, and by analogy the clone 4324229. . .

DETD . . . Ig superfamily class. Expression of 200 gene is many-fold higher in TH1 than in TH2 subpopulations (WO9627603-A1). Modulation of the ***200*** ***gene*** ***product*** may ameliorate a range of T-cell-related disorders. BLASTP searches also show a moderate degree of similarity to kidney injury molecule-1. . .

DETD . . . (ACC:P55083 and U.S. Pat. No. 5,972,654-A, issued Oct. 26, 1999). The human microfibril-associated glycoprotein 4 splice variant (MAG4V) polypeptides and/or ***antibodies*** thereto are disclosed in this patent as being usable to down regulate MAG4V expression and activity. By analogy, Clone 4437909.0.4. . .

DETD . . . receptors or antigens expressed on a selected cell surface,

e.g., by linking the antisense nucleic acid molecules to peptides or ***antibodies*** that bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using.

DET D . . . or derivatives, fragments, analogs or homologs thereof. Also provided are polypeptide fragments suitable for use as immunogens to raise anti-SECX ***antibodies***. In one embodiment, native SECX proteins can be isolated from cells or tissue sources by an appropriate purification scheme using. . .

DET D . . . or inhibiting) cell survival. Moreover, the SECX-immunoglobulin fusion proteins of the invention can be used as immunogens to produce anti-SECX ***antibodies*** in a subject, to purify SECX ligands, and in screening assays to identify molecules that inhibit the interaction of SECX. . .

DET D [0199] Anti-SECX ***Antibodies***

DET D [0200] The invention encompasses ***antibodies*** and ***antibody*** fragments, such as F.sub.ab or (F.sub.ab).sub.2, that bind immunospecifically to any of the polypeptides of the invention.

DET D [0201] An isolated SECX protein, or a portion or fragment thereof, can be used as an immunogen to generate ***antibodies*** that bind SECX using standard techniques for polyclonal and monoclonal ***antibody*** preparation. The full-length SECX protein can be used or, alternatively, the invention provides antigenic peptide fragments of SECX for use. .
. 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30 and encompasses an epitope of SECX such that an ***antibody*** raised against the peptide forms a specific immune complex with SECX. Preferably, the antigenic peptide comprises at least 6, 8, . . .

DET D . . . which regions of a SECX polypeptide are particularly hydrophilic and, therefore, are likely to encode surface residues useful for targeting ***antibody*** production. As a means for targeting ***antibody*** production, hydropathy plots showing regions of hydrophilicity and hydrophobicity may be generated by any method well known in the art, . . .

DET D . . . 26, 28 and 30, or derivatives, fragments, analogs or homologs thereof, may be utilized as immunogens in the generation of ***antibodies*** that immunospecifically-bind these protein components. The term " ***antibody*** " as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that specifically binds (immunoreacts with) an antigen, such as SECX. Such ***antibodies*** include, but are not limited to, polyclonal, monoclonal, chimeric, single chain, F.sub.ab and F.sub.(ab')₂ fragments, and an F.sub.ab expression library. In a specific embodiment, ***antibodies*** to human SECX proteins are disclosed. Various procedures known within the art may be used for the production of polyclonal or monoclonal ***antibodies*** to a SECX protein sequence, or derivative, fragment, analog or homolog thereof. Some of these proteins are discussed below.

DET D [0204] For the production of polyclonal ***antibodies***, various suitable host animals (e.g., rabbit, goat, mouse or other mammal) may be immunized by injection with the native protein, . . . oil emulsions, dinitrophenol, etc.), human adjuvants such as Bacille Calmette-Guerin and Corynebacterium parvum, or similar immunostimulatory agents. If desired, the ***antibody*** molecules directed against SECX can be isolated from the mammal (e.g., from the blood) and further purified by

well known. . .

DETD [0205] The term "monoclonal ***antibody*** " or "monoclonal ***antibody*** composition", as used herein, refers to a population of ***antibody*** molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of SECX. A monoclonal ***antibody*** composition thus typically displays a single binding affinity for a particular SECX protein with which it immunoreacts. For preparation of monoclonal ***antibodies*** directed towards a particular SECX protein, or derivatives, fragments, analogs or homologs thereof, any technique that provides for the production of ***antibody*** molecules by continuous cell line culture may be utilized. Such techniques include, but are not limited to, the hybridoma technique. . . hybridoma technique (see Kozbor, et al., 1983 Immunol Today 4: 72) and the EBV hybridoma technique to produce human monoclonal ***antibodies*** (see Cole, et al, 1985 In: MONOCLONAL ***ANTIBODIES*** AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96). Human monoclonal ***antibodies*** may be utilized in the practice of the present invention and may be produced by using human hybridomas (see Cote, . . . 80: 2026-2030) or by transforming human B-cells with Epstein Barr Virus in vitro (see Cole, et al., 1985 In: MONOCLONAL ***ANTIBODIES*** AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96). Each of the above citations are incorporated herein by reference in. . .

DETD [0206] According to the invention, techniques can be adapted for the production of single-chain ***antibodies*** specific to a SECX protein (see e.g., U.S. Pat. No. 4,946,778). In addition, methodologies can be adapted for the construction. . . of monoclonal F.sub.ab fragments with the desired specificity for a SECX protein or derivatives, fragments, analogs or homologs thereof. Non-human ***antibodies*** can be "humanized" by techniques well known in the art. See e.g., U.S. Pat. No. 5,225,539. ***Antibody*** fragments that contain the idiotypes to a SECX protein may be produced by techniques known in the art including, but not limited to: (i) an F.sub.(ab')₂ fragment produced by pepsin digestion of an ***antibody*** molecule; (ii) an F.sub.ab fragment generated by reducing the disulfide bridges of an F.sub.(ab')₂ fragment; (iii) an F.sub.ab fragment generated by the treatment of the ***antibody*** molecule with papain and a reducing agent and (iv) F.sub.v fragments.

DETD [0207] Additionally, recombinant anti-SECX ***antibodies***, such as chimeric and humanized monoclonal ***antibodies***, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the invention. Such chimeric and humanized monoclonal ***antibodies*** can be produced by recombinant DNA techniques known in the art, for example using methods described in International Application No. . . .

DETD [0208] In one embodiment, methodologies for the screening of ***antibodies*** that possess the desired specificity include, but are not limited to, enzyme-linked immunosorbent assay (ELISA) and other immunologically-mediated techniques known within the art. In a specific embodiment, selection of ***antibodies*** that are specific to a particular domain of a SECX protein is facilitated by generation of hybridomas that bind to the fragment of a SECX protein possessing such a domain. ***Antibodies*** that are specific for an above-described domain within a SECX protein, or derivatives, fragments, analogs or homologs thereof, are also. . .

DETD [0209] Anti-SECX ***antibodies*** may be used in methods known within the art relating to the localization and/or quantitation of a SECX protein (e.g., . . . physiological samples, for use in diagnostic methods, for use in imaging the protein, and the like). In a given embodiment, ***antibodies*** for SECX proteins, or derivatives, fragments, analogs or homologs thereof, that contain the ***antibody*** derived binding domain, are utilized as pharmacologically-active compounds [hereinafter "Therapeutics"].

DETD [0210] An anti-SECX ***antibody*** (e.g., monoclonal ***antibody***) can be used to isolate SECX by standard techniques, such as affinity chromatography or immunoprecipitation. An anti-SECX ***antibody*** can facilitate the purification of natural SECX from cells and of recombinantly produced SECX expressed in host cells. Moreover, an anti-SECX ***antibody*** can be used to detect SECX protein (e.g., in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the SECX protein. Anti-SECX ***antibodies*** can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, e.g., to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (i.e., physically linking) the ***antibody*** to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and. . .

DETD [0237] The SECX nucleic acid molecules, SECX proteins, and anti-SECX ***antibodies*** (also referred to herein as "active compounds") of the invention, and derivatives, fragments, analogs and homologs thereof, can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the nucleic acid molecule, protein, or ***antibody*** and a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion. . .

DETD [0240] Sterile injectable solutions can be prepared by incorporating the active compound (e.g., a SECX protein or anti-SECX ***antibody***) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed. .

DETD . . . be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal ***antibodies*** to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to. . .

DETD [0250] The nucleic acid molecules, proteins, protein homologues, and ***antibodies*** described herein that include extracellular and transmembrane domains and, therefore, can be used in one or more of the following. . .

DETD . . . disorders such as cancer or preclampsia, or any disease or disorder described in Sections 1-14 above). In addition, the anti-SECX ***antibodies*** of the invention can be used to detect and isolate SECX proteins and modulate SECX activity.

DETD . . . (e.g., biotinylation kit, Pierce Chemicals, Rockford, Ill.), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, ***antibodies*** reactive with SECX or target molecules, but which do not interfere with binding of the SECX protein to its target. . . molecule, can be derivatized to the wells of the plate, and unbound target or SECX trapped in the wells by

antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using ***antibodies*** reactive with the SECX or target molecule, as well as enzyme-linked assays that rely on detecting an enzymatic activity associated. . .

DETD . . . been implicated in cardiovascular disorders, including in atherosclerotic plaque formation. Diseases such as cardiovascular disease, including cerebral thrombosis or hemorrhage, ***ischemic*** heart or renal disease, peripheral vascular disease, or thrombosis of other major vessel, and other diseases, including diabetes mellitus, hypertension,. . .

DETD . . . with a monomeric form of a peptide having an activity of another B lymphocyte antigen (e.g., B7-1, B7-3) or blocking ***antibody***), prior to transplantation can lead to the binding of the molecule to the natural ligand(s) on the immune cells without. . .

DETD . . . of inappropriate activation of T cells that are reactive against self tissue and which promote the production of cytokines and auto- ***antibodies*** involved in the pathology of the diseases. Preventing the activation of autoreactive T cells may reduce or eliminate disease symptoms.. . . by disrupting receptor:ligand interactions of B lymphocyte antigens can be used to inhibit T cell activation and prevent production of auto- ***antibodies*** or T cell-derived cytokines which may be involved in the disease process. Additionally, blocking reagents may induce antigen-specific tolerance of. . .

DETD [0337] Assays for T-cell-dependent immunoglobulin responses and isotype switching (which will identify, among others, proteins that modulate T-cell dependent ***antibody*** responses and that affect Th1/Th2 profiles) include, without limitation, those described in: Maliszewski, J Immunol 144:3028-3033, 1990; and Mond and. . .

DETD . . . acute conditions), including without limitation inflammation associated with infection (such as septic shock, sepsis or systemic inflammatory response syndrome (SIRS)), ***ischemia*** -reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine-induced lung injury, inflammatory bowel disease, Crohn's disease or resulting. . .

DETD . . . Methods include, e.g., Northern or Southern blot hybridization (above) or quantitative PCR (below) for measuring SECX nucleic acid levels, and ***antibody*** detection (below), such as ELISA assays and ***antibody*** pull-down assay for measuring SECX polypeptide levels.

DETD [0386] An agent for detecting SECX protein is an ***antibody*** capable of binding to SECX protein, preferably an ***antibody*** with a detectable label. ***Antibodies*** can be polyclonal, or more preferably, monoclonal. An intact ***antibody***, or a fragment thereof (e.g., Fab or F(ab').sub.2) can be used. The term "labeled", with regard to the probe or ***antibody***, is intended to encompass direct labeling of the probe or ***antibody*** by coupling (i.e., physically linking) a detectable substance to the probe or ***antibody***, as well as indirect labeling of the probe or ***antibody*** by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary ***antibody*** using a fluorescently labeled secondary ***antibody*** and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin. The term. .

. include Southern hybridizations. Furthermore, in vivo techniques for detection of SECX protein include introducing into a subject a labeled anti-SECX ***antibody***. For example, the ***antibody*** can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging. . .

DETD . . . described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or ***antibody*** reagent described herein, which may be conveniently used, e.g., in clinical settings to diagnose patients exhibiting symptoms or family history. . .

DETD . . . be utilized include, but are not limited to, (i) an aforementioned peptide, or analogs, derivatives, fragments or homologs thereof; (ii) ***antibodies*** to an aforementioned peptide; (iii) nucleic acids encoding an aforementioned peptide; (iv) administration of antisense nucleic acid and nucleic acids. . . 1989, Science 244:1288-1292); or (v) modulators (i.e., inhibitors, agonists and antagonists, including additional peptide mimetic of the invention or ***antibodies*** specific to a peptide of the invention) that alter the interaction between an aforementioned peptide and its binding partner.

DETD . . . inhibits one or more SECX protein activity. Examples of such inhibitory agents include antisense SECX nucleic acid molecules and anti-SECX ***antibodies***. These modulatory methods can be performed in vitro (e.g., by culturing the cell with the agent) or, alternatively, in vivo. . .

DETD . . . supernatant were harvested 72 hours after transfection and examined for h11753149 expression by Western blotting (reducing conditions) with an anti-V5 ***antibody***. FIG. 15 shows that h11753149 is expressed as a protein secreted by 293 cells that is broadly distributed around 64. . .

DETD . . . supernatant were harvested 72 hours after transfection and examined for h4437909 expression by Western blotting (reducing conditions) with an anti-V5 ***antibody***. FIG. 16 shows that h4437909 is expressed in 293 cells as three discrete secreted protein bands of 16, 40, and. . .

DETD . . . according to the manufacturer's instructions. After induction, total cells were harvested, and proteins were analyzed by Western blotting using anti-HisGly ***antibody*** (Invitrogen, Carlsbad, Calif.). FIG. 17 shows h4437909 was expressed as a 34 kDa protein in E. coli cells. This corresponds. . .

CLM What is claimed is:

21. An ***antibody*** that binds immunospecifically to the polypeptide of claim 1.

22. The ***antibody*** of claim 21, wherein said ***antibody*** is a monoclonal ***antibody***.

23. The ***antibody*** of claim 21, wherein the ***antibody*** is a humanized ***antibody***.

24. An ***antibody*** that binds immunospecifically to the polypeptide of claim 5.

25. The ***antibody*** of claim 24, wherein said ***antibody*** is a monoclonal ***antibody***.

26. The ***antibody*** of claim 24, wherein the ***antibody*** is a humanized ***antibody*** .

27. A method for determining the presence or amount of the polypeptide of claim 1 in a sample, the method comprising: (a) providing the sample; (b) contacting the sample with an ***antibody*** that binds immunospecifically to a polypeptide of claim 1; and (c) determining the presence or amount of ***antibody*** bound to said polypeptide, thereby determining the presence or amount of polypeptide in said sample.

. . . polypeptide of claim 5 in a sample, the method comprising: (a) providing the sample; (b) contacting the sample with an ***antibody*** that binds immunospecifically to a polypeptide of claim 5; and (c) determining the presence or amount of ***antibody*** bound to said polypeptide, thereby determining the presence or amount of polypeptide in said sample.

51. A pharmaceutical composition comprising the ***antibody*** of claim 21 and a pharmaceutically acceptable carrier.

52. A pharmaceutical composition comprising the ***antibody*** of claim 24 and a pharmaceutically acceptable carrier.

. . . wherein said therapeutic is selected from the group consisting of a SECX polypeptide, a SECX nucleic acid, and a SECX ***antibody*** .

70. A method of treating a pathological state in a mammal, the method comprising administering to the mammal the ***antibody*** of claim 21 in an amount sufficient to alleviate the pathological state.

71. A method of treating a pathological state in a mammal, the method comprising administering to the mammal the ***antibody*** of claim 24 in an amount sufficient to alleviate the pathological state.

L2 ANSWER 6 OF 7 USPATFULL on STN

AN 2002:301172 USPATFULL

TI Novel amino acid sequences for human microfibril glycoprotein 4-like polypeptides

IN Fernandes, Elma, Branford, CT, UNITED STATES

Vernet, Corine, Gainesville, FL, UNITED STATES

Shimkets, Richard A., West Haven, CT, UNITED STATES

PA CuraGen Corporation, New Haven, CT, 06511 (U.S. corporation)

PI US 2002168716 A1 20021114

AI US 2001-966546 A1 20010926 (9)

RLI Continuation of Ser. No. US 2000-544511, filed on 6 Apr 2000, ABANDONED

PRAI US 2000-186592P 20000303 (60)

US 1999-128514P 19990409 (60)

DT Utility

FS APPLICATION

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CLMN Number of Claims: 86

ECL Exemplary Claim: 1
DRWN 42 Drawing Page(s)
LN.CNT 5335

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This application is drawn to novel amino acid sequences for mammalian polypeptides that have sequence similarity to human microfibril-associated glycoprotein 4, and processes for preparing the same. The clone identified has a nucleic acid sequence that is 1099 nucleotides long, contains an open reading frame from nucleotides 83-85 to 890-892, and encodes a novel polypeptide that is 269 amino acids long.

SUMM [0003] The invention relates to polynucleotides and polypeptides encoded by such polynucleotides, as well as vectors, host cells, ***antibodies*** and recombinant methods for producing the polypeptides and polynucleotides.

SUMM [0012] Also included in the invention is an ***antibody*** which selectively binds to a SECX polypeptide.

SUMM . . . nucleic acid in a sample from a mammal, e.g., a human, by contacting a sample from the mammal with an ***antibody*** which selectively binds to one of the herein described polypeptides, and detecting the formation of reaction complexes including the ***antibody*** and the polypeptide in the sample. Detecting the formation of complexes in the sample indicates the presence of the polypeptide in the sample. Methods for measurements of ***antibody*** reaction complex concentrations are well known in the art. Methods for detecting and quantitating nucleic acids include hybridization and TaqMan.TM.. . .

SUMM [0019] Alternatively, the mammal may be treated by administering an ***antibody*** as herein described in an amount sufficient to alleviate the pathological condition.

DETD . . . having 180 residues (WO9932632-A1, published Jul. 1, 1999). The EDIRF-like DNA and protein sequences (e.g., Clone 2191999) and their homologues, ***antibodies*** (Ab) specific for EDIRF-like protein, and other modulators may be used: (i) in screening and detection assays, e.g. for chromosome. . .

DETD . . . fragment of human limbic system associated membrane protein (LAMP; PCT Publication WO9630052-A1, published Oct. 3, 1996). LAMP is a self-binding, ***antibody*** -like cell surface adhesion protein involved in formation of connections between adjacent neurons. LAMP protein, and by analogy the clone 4324229. . .

DETD . . . Ig superfamily class. Expression of 200 gene is many-fold higher in TH1 than in TH2 subpopulations (WO9627603-A1). Modulation of the ***200*** ***gene*** ***product*** may ameliorate a range of T-cell-related disorders. BLASTP searches also show a moderate degree of similarity to kidney injury molecule-1. . .

DETD . . . (ACC:P55083 and U.S. Pat. No. 5,972,654-A, issued Oct. 26, 1999). The human microfibril-associated glycoprotein 4 splice variant (MAG4V) polypeptides and/or ***antibodies*** thereto are disclosed in this patent as being usable to down regulate MAG4V expression and activity. By analogy, Clone 4437909.0.4. . .

DETD . . . receptors or antigens expressed on a selected cell surface, e.g., by linking the antisense nucleic acid molecules to peptides or ***antibodies*** that bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using.

DETD . . . or derivatives, fragments, analogs or homologs thereof. Also

provided are polypeptide fragments suitable for use as immunogens to raise anti-SECX ***antibodies***. In one embodiment, native SECX proteins can be isolated from cells or tissue sources by an appropriate purification scheme using. . .

DETD . . . or inhibiting) cell survival. Moreover, the SECX-immunoglobulin fusion proteins of the invention can be used as immunogens to produce anti-SECX ***antibodies*** in a subject, to purify SECX ligands, and in screening assays to identify molecules that inhibit the interaction of SECX. . .

DETD [0198] Anti-SECX ***Antibodies***

DETD [0199] The invention encompasses ***antibodies*** and ***antibody*** fragments, such as F.sub.ab or (F.sub.ab).sub.2, that bind immunospecifically to any of the polypeptides of the invention.

DETD [0200] An isolated SECX protein, or a portion or fragment thereof, can be used as an immunogen to generate ***antibodies*** that bind SECX using standard techniques for polyclonal and monoclonal ***antibody*** preparation. The full-length SECX protein can be used or, alternatively, the invention provides antigenic peptide fragments of SECX for use. . . 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30 and encompasses an epitope of SECX such that an ***antibody*** raised against the peptide forms a specific immune complex with SECX. Preferably, the antigenic peptide comprises at least 6, 8, . . .

DETD . . . which regions of a SECX polypeptide are particularly hydrophilic and, therefore, are likely to encode surface residues useful for targeting ***antibody*** production. As a means for targeting ***antibody*** production, hydropathy plots showing regions of hydrophilicity and hydrophobicity may be generated by any method well known in the art, . . .

DETD . . . 26, 28 and 30, or derivatives, fragments, analogs or homologs thereof, may be utilized as immunogens in the generation of ***antibodies*** that immunospecifically-bind these protein components. The term " ***antibody*** " as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that specifically binds (immunoreacts with) an antigen, such as SECX. Such ***antibodies*** include, but are not limited to, polyclonal, monoclonal, chimeric, single chain, F.sub.ab and F.sub.(ab')₂ fragments, and an F.sub.ab expression library. In a specific embodiment, ***antibodies*** to human SECX proteins are disclosed. Various procedures known within the art may be used for the production of polyclonal or monoclonal ***antibodies*** to a SECX protein sequence, or derivative, fragment, analog or homolog thereof. Some of these proteins are discussed below.

DETD [0203] For the production of polyclonal ***antibodies***, various suitable host animals (e.g., rabbit, goat, mouse or other mammal) may be immunized by injection with the native protein, . . . oil emulsions, dinitrophenol, etc.), human adjuvants such as Bacille Calmette-Guerin and Corynebacterium parvum, or similar immunostimulatory agents. If desired, the ***antibody*** molecules directed against SECX can be isolated from the mammal (e.g., from the blood) and further purified by well known. . .

DETD [0204] The term "monoclonal ***antibody*** " or "monoclonal ***antibody*** composition", as used herein, refers to a population of ***antibody*** molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of

SECX. A monoclonal ***antibody*** composition thus typically displays a single binding affinity for a particular SECX protein with which it immunoreacts. For preparation of monoclonal ***antibodies*** directed towards a particular SECX protein, or derivatives, fragments, analogs or homologs thereof, any technique that provides for the production of ***antibody*** molecules by continuous cell line culture may be utilized. Such techniques include, but are not limited to, the hybridoma technique. . . hybridoma technique (see Kozbor, et al., 1983 Immunol Today 4: 72) and the EBV hybridoma technique to produce human monoclonal ***antibodies*** (see Cole, et al., 1985 In: MONOCLONAL ***ANTIBODIES*** AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96). Human monoclonal ***antibodies*** may be utilized in the practice of the present invention and may be produced by using human hybridomas (see Cote, . . .

DETD [0205] According to the invention, techniques can be adapted for the production of single-chain ***antibodies*** specific to a SECX protein (see e.g., U.S. Pat. No. 4,946,778). In addition, methodologies can be adapted for the construction. . . of monoclonal F.sub.ab fragments with the desired specificity for a SECX protein or derivatives, fragments, analogs or homologs thereof. Non-human ***antibodies*** can be "humanized" by techniques well known in the art. See e.g., U.S. Pat. No. 5,225,539. ***Antibody*** fragments that contain the idiotypes to a SECX protein may be produced by techniques known in the art including, but not limited to: (i) an F.sub.(ab')₂ fragment produced by pepsin digestion of an ***antibody*** molecule; (ii) an F.sub.ab fragment generated by reducing the disulfide bridges of an F.sub.(ab')₂ fragment; (iii) an F.sub.ab fragment generated by the treatment of the ***antibody*** molecule with papain and a reducing agent and (iv) F.sub.v fragments.

DETD [0206] Additionally, recombinant anti-SECX ***antibodies***, such as chimeric and humanized monoclonal ***antibodies***, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the invention. Such chimeric and humanized monoclonal ***antibodies*** can be produced by recombinant DNA techniques known in the art, for example using methods described in International Application No. . . .

DETD [0207] In one embodiment, methodologies for the screening of ***antibodies*** that possess the desired specificity include, but are not limited to, enzyme-linked immunosorbent assay (ELISA) and other immunologically-mediated techniques known within the art. In a specific embodiment, selection of ***antibodies*** that are specific to a particular domain of a SECX protein is facilitated by generation of hybridomas that bind to the fragment of a SECX protein possessing such a domain. ***Antibodies*** that are specific for an above-described domain within a SECX protein, or derivatives, fragments, analogs or homologs thereof, are also. . .

DETD [0208] Anti-SECX ***antibodies*** may be used in methods known within the art relating to the localization and/or quantitation of a SECX protein (e.g., . . . physiological samples, for use in diagnostic methods, for use in imaging the protein, and the like). In a given embodiment, ***antibodies*** for SECX proteins, or derivatives, fragments, analogs or homologs thereof, that contain the ***antibody*** derived binding domain, are utilized as pharmacologically-active compounds [hereinafter "Therapeutics"].

DETD [0209] An anti-SECX ***antibody*** (e.g., monoclonal

antibody) can be used to isolate SECX by standard techniques, such as affinity chromatography or immunoprecipitation. An anti-SECX ***antibody*** can facilitate the purification of natural SECX from cells and of recombinantly produced SECX expressed in host cells. Moreover, an anti-SECX ***antibody*** can be used to detect SECX protein (e.g., in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the SECX protein. Anti-SECX ***antibodies*** can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, e.g., to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (i.e., physically linking) the ***antibody*** to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and. . .

DETD [0236] The SECX nucleic acid molecules, SECX proteins, and anti-SECX ***antibodies*** (also referred to herein as "active compounds") of the invention, and derivatives, fragments, analogs and homologs thereof, can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the nucleic acid molecule, protein, or ***antibody*** and a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion. . .

DETD [0239] Sterile injectable solutions can be prepared by incorporating the active compound (e.g., a SECX protein or anti-SECX ***antibody***) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed. . .

DETD . . . be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal ***antibodies*** to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to. . .

DETD [0249] The nucleic acid molecules, proteins, protein homologues, and ***antibodies*** described herein that include extracellular and transmembrane domains and, therefore, can be used in one or more of the following. . .

DETD . . . disorders such as cancer or preclampsia, or any disease or disorder described in Sections 1-14 above). In addition, the anti-SECX ***antibodies*** of the invention can be used to detect and isolate SECX proteins and modulate SECX activity.

DETD . . . (e.g., biotinylation kit, Pierce Chemicals, Rockford, Ill.), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, ***antibodies*** reactive with SECX or target molecules, but which do not interfere with binding of the SECX protein to its target. . . molecule, can be derivatized to the wells of the plate, and unbound target or SECX trapped in the wells by ***antibody*** conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using ***antibodies*** reactive with the SECX or target molecule, as well as enzyme-linked assays that rely on detecting an enzymatic activity associated. . .

DETD . . . been implicated in cardiovascular disorders, including in atherosclerotic plaque formation. Diseases such as cardiovascular disease, including cerebral thrombosis or hemorrhage, ***ischemic*** heart or renal disease, peripheral vascular disease, or thrombosis of

other major vessel, and other diseases, including diabetes mellitus, hypertension, . . .

DETD . . . with a monomeric form of a peptide having an activity of another B lymphocyte antigen (e.g., B7-1, B7-3) or blocking ***antibody***), prior to transplantation can lead to the binding of the molecule to the natural ligand(s) on the immune cells without. . .

DETD . . . of inappropriate activation of T cells that are reactive against self tissue and which promote the production of cytokines and auto- ***antibodies*** involved in the pathology of the diseases. Preventing the activation of autoreactive T cells may reduce or eliminate disease symptoms.. . . by disrupting receptor:ligand interactions of B lymphocyte antigens can be used to inhibit T cell activation and prevent production of auto- ***antibodies*** or T cell-derived cytokines which may be involved in the disease process. Additionally, blocking reagents may induce antigen-specific tolerance of. . .

DETD [0336] Assays for T-cell-dependent immunoglobulin responses and isotype switching (which will identify, among others, proteins that modulate T-cell dependent ***antibody*** responses and that affect Th1/Th2 profiles) include, without limitation, those described in: Maliszewski, J Immunol 144:3028-3033, 1990; and Mond and. . .

DETD . . . acute conditions), including without limitation inflammation associated with infection (such as septic shock, sepsis or systemic inflammatory response syndrome (SIRS)), ***ischemia*** -reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine-induced lung injury, inflammatory bowel disease, Crohn's disease or resulting. . .

DETD . . . Methods include, e.g., Northern or Southern blot hybridization (above) or quantitative PCR (below) for measuring SECX nucleic acid levels, and ***antibody*** detection (below), such as ELISA assays and ***antibody*** pull-down assay for measuring SECX polypeptide levels.

DETD [0384] An agent for detecting SECX protein is an ***antibody*** capable of binding to SECX protein, preferably an ***antibody*** with a detectable label. ***Antibodies*** can be polyclonal, or more preferably, monoclonal. An intact ***antibody***, or a fragment thereof (e.g., Fab or F(ab').sub.2) can be used. The term "labeled", with regard to the probe or ***antibody***, is intended to encompass direct labeling of the probe or ***antibody*** by coupling (i.e., physically linking) a detectable substance to the probe or ***antibody***, as well as indirect labeling of the probe or ***antibody*** by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary ***antibody*** using a fluorescently labeled secondary ***antibody*** and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin. The term. . . include Southern hybridizations. Furthermore, in vivo techniques for detection of SECX protein include introducing into a subject a labeled anti-SECX ***antibody***. For example, the ***antibody*** can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging. . .

DETD . . . described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or ***antibody*** reagent described herein, which may be conveniently used, e.g., in clinical settings to diagnose patients

exhibiting symptoms or family history. . .

DETD . . . be utilized include, but are not limited to, (i) an aforementioned peptide, or analogs, derivatives, fragments or homologs thereof; (ii) ***antibodies*** to an aforementioned peptide; (iii) nucleic acids encoding an aforementioned peptide; (iv) administration of antisense nucleic acid and nucleic acids. . . 1989, Science 244: 1288-1292); or (v) modulators (i.e., inhibitors, agonists and antagonists, including additional peptide mimetic of the invention or ***antibodies*** specific to a peptide of the invention) that alter the interaction between an aforementioned peptide and its binding partner.

DETD . . . inhibits one or more SECX protein activity. Examples of such inhibitory agents include antisense SECX nucleic acid molecules and anti-SECX ***antibodies***. These modulatory methods can be performed in vitro (e.g., by culturing the cell with the agent) or, alternatively, in vivo. . .

DETD . . . supernatant were harvested 72 hours after transfection and examined for h11753149 expression by Western blotting (reducing conditions) with an anti-V5 ***antibody***. FIG. 15 shows that h11753149 is expressed as a protein secreted by 293 cells that is broadly distributed around 64. . .

DETD . . . supernatant were harvested 72 hours after transfection and examined for h4437909 expression by Western blotting (reducing conditions) with an anti-V5 ***antibody***. FIG. 16 shows that h4437909 is expressed in 293 cells as three discrete secreted protein bands of 16, 40, and. . .

DETD . . . according to the manufacturer's instructions. After induction, total cells were harvested, and proteins were analyzed by Western blotting using anti-HisGly ***antibody*** (Invitrogen, Carlsbad, Calif.). FIG. 17 shows h4437909 was expressed as a 34 kDa protein in E. coli cells. This corresponds. . .

CLM What is claimed is:

21. An ***antibody*** that binds immunospecifically to the polypeptide of claim 1.

22. The ***antibody*** of claim 21, wherein said ***antibody*** is a monoclonal ***antibody***.

23. The ***antibody*** of claim 21, wherein the ***antibody*** is a humanized ***antibody***.

24. An ***antibody*** that binds immunospecifically to the polypeptide of claim 5.

25. The ***antibody*** of claim 24, wherein said ***antibody*** is a monoclonal ***antibody***.

26. The ***antibody*** of claim 24, wherein the ***antibody*** is a humanized ***antibody***.

27. A method for determining the presence or amount of the polypeptide of claim 1 in a sample, the method comprising: (a) providing the sample; (b) contacting the sample with an ***antibody*** that binds immunospecifically to a polypeptide of claim 1; and (c) determining the presence or amount of ***antibody*** bound to said polypeptide,

thereby determining the presence or amount of polypeptide in said sample.

. . . polypeptide of claim 5 in a sample, the method comprising: (a) providing the sample; (b) contacting the sample with an ***antibody*** that binds immunospecifically to a polypeptide of claim 5; and (c) determining the presence or amount of ***antibody*** bound to said polypeptide, thereby determining the presence or amount of polypeptide in said sample.

. . . preventing a SECX-associated disorder, said method comprising administering to a subject in which such treatment or prevention is desired the ***antibody*** of claim 21 in an amount sufficient to treat or prevent said SECX-associated disorder in said subject.

51. A pharmaceutical composition comprising the ***antibody*** of claim 21 and a pharmaceutically acceptable carrier.

52. A pharmaceutical composition comprising the ***antibody*** of claim 24 and a pharmaceutically acceptable carrier.

. . . wherein said therapeutic is selected from the group consisting of a SECX polypeptide, a SECX nucleic acid, and a SECX ***antibody*** .

70. A method of treating a pathological state in a mammal, the method comprising administering to the mammal the ***antibody*** of claim 21 in an amount sufficient to alleviate the pathological state.

71. A method of treating a pathological state in a mammal, the method comprising administering to the mammal the ***antibody*** of claim 24 in an amount sufficient to alleviate the pathological state. What is claimed is:

78. The method of claim 77, wherein said SEQ ID NO:26 polypeptide is detected using an ***antibody*** .

L2 ANSWER 7 OF 7 USPATFULL on STN

AN 2002:66849 USPATFULL

TI Method for diagnosing and alleviating the symptoms of chronic fatigue syndrome

IN Lerner, A. Martin, Birmingham, MI, UNITED STATES

PI US 2002037501 A1 20020328

US 6399622 B2 20020604

AI US 2001-971291 A1 20011003 (9)

RLI Continuation-in-part of Ser. No. US 2000-663729, filed on 15 Sep 2000,
PENDING Continuation-in-part of Ser. No. US 1998-177942, filed on 23 Oct
1998, GRANTED, Pat. No. US 6258818 Division of Ser. No. US 1997-802776,
filed on 18 Feb 1997, GRANTED, Pat. No. US 5872123

DT Utility

FS APPLICATION

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CLMN Number of Claims: 2

ECL Exemplary Claim: 1

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method for alleviating chronic fatigue syndrome with the administration of antiviral agents. Based on clinical tests, chronic fatigue syndrome is a persistent herpes virus infection including incomplete virus multiplication and thus administration of antiviral agents are shown to alleviate the symptoms associated with the disorder. Based on therapeutic trials, patients receiving the recommended antiviral treatment, have experienced significant reduction or elimination of the symptoms associated with chronic fatigue syndrome. A method of diagnosis of the chronic fatigue syndrome is further disclosed.

SUMM . . . is the basis of the CFS. The serologic evidence of EBV and HCMV is obtained by studying the level of ***antibodies*** of EBV and HCMV to detect the presence of active and persistent multiplication of either or both of the viruses.. . .

DRWD [0023] FIG. 3 is a graph depicting a 39-year old female patient's HCMV ***antibody*** titer levels following the administration of antiviral treatment; and

DRWD [0024] FIG. 4 is a graph depicting a 47-year old male patient's ***antibody*** titer levels following the administration of antiviral treatment.

DETD . . . presence of EBV and/or HCMV in CFS sufferers. Our research has further indicated for CFS sufferers the existence of IgM ***antibodies*** to the EBV viral capsid antigen (VCA) or EBV ***antibodies*** to diffuse early antigen (EA), the latter depicting EBV transactivators for the lytic cycle indicating current virus multiplication. In CFS sufferers, there may additionally or alternatively be a significant IgG immunoreactive ***antibody*** (ELISA) to HCMV, with/without an IgM (ELISA) ***antibody*** titer to HCMV.

DETD . . . that previous seroepidemiologic studies attempting to show a singular virologic causation to CFS including singular searches for EBV or HCMV ***antibodies*** would have naturally yielded uniformly negative results. At least fifteen different viruses, bacteria and parasites have been previously suspected as. . .

DETD [0036] The herpes virus genome is capable of making over ***200*** ***gene*** ***products*** and 200 separate proteins. In FIG. 1c the latent double-stranded DNA has opened--is in its linear position and ready to. . .

DETD . . . of viral enzymes and factors necessary for viral assembly. A complete herpes virus virion 10 is depicted in FIG. 1d. ***Antibodies*** to structural glycoprotein, for instance gB and gH of human cytomegalovirus, are produced. In the case of incomplete virus replication,. . . UL44 and UL57) reach the extracellular space and are thus available to the human host immune system so that specific ***antibodies*** may be produced to these nonstructural gene products, UL44 and UL57. These nonstructural gene products are behind the virus bilayer 16 and are not exposed to ***antibody*** production when whole complete virus multiplication occurs.

DETD . . . contradictory. Just like CFS, herpes simplex virus encephalitis (HSVE) boggled the medical community and etiologic identification due to the rising ***antibodies*** in serum which may or may not be present at a given time. Diagnosis of HSVE required isolation of herpes. . .

DETD [0054] Table 1 describes the ***antibody*** response to whole virus infection which results in ***antibodies*** to viral structural epitopes and a response to nonstructural viral epitopes such as would be seen in incomplete viral replication.. . .

DETD [0055] This Copalis.RTM. method to CM.sub.2 and p52 measures IgM ***antibodies*** to HCMV non-structural gene products. The non-structural gene products are UL44, UL57. These gene products have been produced in E-coli.. . . These are nonstructural gene products which are extruded from a cell with incomplete human cytomegalovirus multiplication. The Copalis.RTM. method measures ***antibodies*** to these two gene products with the purified antigens produced in E-coli. Table 1 shows the types of herpes virus infection with ***antibody*** responses to both structural and nonstructural viral epitopes.

TABLE 1

HERPES VIRUS INFECTION

Structural Viral Epitopes Non-Structural Viral
(e.g. HCMVgB, HCMVgH). . .

DETD [0056] In virulent virus infection, the infected cell is destroyed. Cellular inflammation in the classical sense results. Specific ***antibodies*** to structural epitopes of human cytomegalovirus for instance gB and gH result. This results in IgM and IgG ***antibodies***. The IgM ***antibody***, of course, is short-lived usually less than six weeks, and the IgG ***antibody*** is long-lived. In classical virulent infection no ***antibodies*** would be produced to nonstructural viral epitopes, hereinafter referred to as p52 (to UL44 and UL57) and CM.sub.2 (UL57). In latent infection there is no complete virus produced. There is no IgM to structural epitopes gB and gH ***antibody***. IgG ***antibody***, of course, is present because there is past infection and, of course, there is no ***antibody*** to p52 or CM.sub.2. In incomplete viral multiplication, no IgM ***antibodies*** to structural epitopes are produced. Of course, IgG ***antibodies*** are stable and present. In incomplete viral multiplication, IgM ***antibodies*** are produced to nonstructural epitopes (Table 1). Finally in mixed virulent and incomplete (or complete and incomplete viral replication/multiplication), one may have IgM ***antibodies*** and IgG ***antibodies*** to structural epitopes and IgM ***antibodies*** to nonstructural epitopes.

DETD . . . one of three antigens (p52, CM.sub.2, and CMV viral particle (VP)) are dried in the reaction cup. Early phase IgM ***antibodies*** are detected by both the CM.sub.2 and the p52 antigens. ***Antibody*** response to the CM.sub.2 antigen but not to the p52 antigen continues through the convalescent stage of acute infection. ***Antibodies*** to the VP may appear during acute infection but shall persist during life. Reactivity just (sucrose-density purified tissue culture produced complete HCMV cytomegalovirus) to the VP is characteristic of prior infection (indicative of immune status). ***Antibody*** detection, made by the Copalis.RTM. Immunoassay System, is reported qualitatively for the IgM analytes and semi-quantitatively for the total analyte.

DETD [0059] The Copalis.RTM. CMVplex ***Antibody*** Assay is based on the principle of ***antibody*** dependent particle aggregation as measured by the amount of light scattered. A typical ***antibody*** reactivity pattern (IgM and/or IgG) in the patient sample allows

diagnosis of the infection as seronegative, acute or past infections..

DET D [0061] Copalis.RTM. CMVplex ***Antibody*** Assay

DET D [0066] The Copalis.RTM. light scattering technique thus allows for detection and precise measurement of ***antibodies*** to HCMV. It should be noted that p52 and CM.sub.2 ***antibody*** may also be detected by ELISA methods.

DET D [0067] The EA ***antibody*** is measured in the diagnosis of the chronic fatigue syndrome due to Epstein-Barr virus infection. This polymeric EA ***antibody*** to diffuse early antigen products is an ***antibody*** to as many as 12 nonstructural gene products, which are transactivators for the lytic cycle of Epstein-Barr virus so that the paradigm of complete and incomplete viral multiplication in ***antibodies*** in structural and nonstructural epitopes outlined for human cytomegalovirus relates to human Epstein-Barr virus as well. Both, of course, are. . .

DET D . . . by the therapy. One indicator of an improvement in EBV-isolated CFS patients is a decrease in the level of IgM ***antibodies*** to the VCA for EBV. Generally, the therapy duration is proportional to the intensity of the CFS manifestation. Accordingly, following. . .

DET D [0094] Serum ***antibody*** titers to Epstein-Barr virus (EBV) and cytomegalovirus (HCMV) were assayed in a consecutive case series of 98 patients with the. . . CFS patients had evidence of a cardiomyopathy documented by the presence of abnormal left ventricular dynamics at stress/rest (MUGA) tests. ***Antibodies*** to Epstein-Barr virus (EBV) immunofluorescent total early antigens (EA-D) and cytomegalovirus (HCMV) IgM and IgG enzyme immunoassays (ELISA) were measured. The prevalence of these ***antibodies*** was compared to that in a non-CFS group of control persons from the same area. Approximately 50% of CFS and non-CFS patients had concurrent EBV multiplication when tested by the presence of an elevated EA ***antibody*** titer to the (EAD) diffuse complex which neutralizes EBV encoded DNA polymerase. Elevated IgM HCMV ***antibodies*** were uncommon (<10%) in all groups. Approximately 40% of the CFS patients and non-CFS controls had no IgG HCMV ***antibody***. These data are consistent with our understanding that CFS is a persistent cardiomyopathic infection caused by EBV or HCMV, or. . .

DET D . . . serologic evidence that the human cardiac myocyte harbors EBV, HCMV or both viruses in CFS patients, we assayed EBV serum ***antibody*** total Early Antigen (EA) and IgM ***antibodies*** to the viral capsid antigen (VCA) and HCMV IgM and IgG serum ***antibodies***.

DET D . . . of another important herpes virus infection, namely, herpes simplex virus (HSV) encephalitis was not possible by tests of specific HSV ***antibodies*** in serum. Brain biopsy with isolation of HSV, type 1 or type 2 from affected sites was necessary for diagnosis.. . .

DET D . . . radioisotopic gated blood pool method. Rest/stress myocardial perfusion studies (thallium 201 or TC-99m sestamibi) or, as necessary, cardiac catheterizations excluded ***ischemic*** cardiomyopathy in each case. CFS patient's demographic data were similar to other series. The mean age here was 42.3+-.10.6 years,. . .

DET D [0101] ***Antibody*** Titers.

DET D [0102] At the initial clinical visit of each CFS patient, EBV total early antigens (EA) immunofluorescent ***antibody*** titers (Roche Laboratories, Columbus, Ohio) and cytomegalovirus (HCMV) IgM enzyme

immunoassays (ELISA) (Detroit Biomedical Laboratories, Detroit, Mich.) and HCMV ELISA. . . IgG and IgM species onto the solid phase by anti-human immunoglobulin followed by the addition of antigen and labeling the ***antibodies***. With regard to IgM assays, this ELISA method reduces the potential interference of rheumatoid factor. During the EBV infectious lytic. . . (EA-R) complex. We assayed the EA-D. The 52/50 KD.sub.a EA-D protein complex neutralizes EBV encoded DNA polymerase activity. EBV EA ***antibody*** indicates recent EBV multiplication (e.g. within 90 days). Likewise, HCMV ELISA IgM enzyme immunoassays indicate recent virus multiplication. A positive. . . IgG titer does not differentiate concurrent from past HCMV infection. As a control group to the CFS patients, similar serum ***antibody*** titers were measured in 20 random well individuals from the same area. They were 60% women with a mean age. . .

DETD [0103] The prevalence of EBV EA ***antibodies*** were similar in all CFS patients, both those with and those without abnormal left ventricular dynamics. The prevalence of these EBV ***antibodies*** were also the same in non-CFS well persons. Approximately one-half of each of the groups, CFS and non-CFS, had concurrent persistent multiplication of EBV as indicated by an EA ***antibody*** titer of .gtoreq.10. Similarly, IgG HCMV ***antibodies*** were present in approximately 60% of each group. However, HCMV, IgM ***antibodies*** were uncommon (<10%).

DETD . . . was demonstrated, were treated and studied over a six month period. EBV active infection was documented by EBV VCA IgM ***antibodies*** and/or EBV EA elevated ***antibody*** titers. All of these patients had their energy levels reduced by 50% or more and were partially or entirely non-functional,. . .

DETD [0114] During the initial and final clinical visits, each CFS patient was studied to determine the IgM ***antibodies*** to the EBV viral capsid antigen (VCA), total early antigens (EA), and enzyme immunoassays (ELISA) to HCMV IgM and HCMV ELISA IgG titers. An IgM EBV VCA ***antibody*** titer indicates infectious lytic EBV multiplication within the most recent 3 month period. During the EBV infectious lytic cycle, antigen. . . and a cytoplasmic restricted EAR complex (EAR). As a result, each patient's EAD was assayed. The presence of EBV EA ***antibody*** indicates current EBV multiplication. Likewise, an elevated cytomegalovirus or Epstein-Barr virus IgM ***antibody*** indicates recent EBV or HCMV multiplication, respectively. The EBV VCA ***antibody*** IgM titers were done by the ELISA method. As a control group, similar serum ***antibody*** titers were measured in 20 non-CFS individuals. In the non-CFS control group, the mean HCMV IgG titer was 240.

DETD . . . VCA, IgM titers in five out of ten. The remainder of the EBV suspected CFS patients had elevated EBV EA ***antibody*** titers. At the completion of the trial, two out of ten continued to have VCA IgM positive titers. At the beginning of the trial, seven out of ten had positive EA ***antibody*** titers (.gtoreq.10). At the completion of the trial, eight out of ten continued to have positive EA ***antibody*** titers. This population of ten patients was characterized by little to no HCMV experience. None had a HCMV IgM titer;. . .

DETD . . . to assess the possible efficacy of ganciclovir treatment on a subset of CFS patients with (1) high HCMV IgG ELISA ***antibody*** titers; (2) minimal/no serologic evidence of concurrent EBV

multiplication; and (3) oscillating ECG abnormalities at Holter monitoring.

DETD . . . HCMV ELISA IgG titers, as well as EBV capsid antigen (VCA), ELISA IgM and EBV total early antigens (EA) immunofluorescent ***antibody*** titers were assayed. Additionally, buffy coats, urine and myocardial biopsies were tested for infectious HCMV. As a control of the occurrence of HCMV and EBV virus ***antibodies*** in normal non-CFS persons, residing in the same area, 20 random individuals were tested for HCMV ***antibodies*** and for EBV ***antibodies***. Holter monitoring, MUGA studies and endomyocardial biopsies in these patients were done.

DETD [0138] HCMV and EBV ***Antibody*** Titers.

DETD [0139] Eight of the patients had high HCMV ELISA IgG ***antibody*** titers with a mean of 322u. Two patients had positive IgM HCMV ***antibody*** titers. Ten of the 13 patients had little to no evidence of EBV multiplication as tested by an elevated ***antibody*** titer to EA.

DETD . . . normal. At resting 12-lead ECG showed an inverted T-wave in standard lead III, but was otherwise normal. An ELISA IgM ***antibody*** titer to HCMV was positive, while Epstein-Barr virus ***antibody*** tests were negative. Holter monitoring showed oscillating abnormal flat or inverted T-waves appearing with the onset of sinus tachycardias, and. . .

DETD [0146] A myocardial sestamibi perfusion rest/stress test showed reversible ***ischemia*** of the anterior, apical and inferior walls, but at cardiac catheterization, the coronary arteries were patent. A stress MUGA study. . .

DETD [0149] Based upon these research results, it is understood that CFS patients with a significant ELISA IgG HCMV ***antibody*** titer, greater than 120 units, with or without the presence of an IgM HCMV ELISA ***antibody*** titer plus an absence of EBV VCA IgM ***antibody*** titer along with an EBV EA ***antibody*** titer less than 40 describes a group of CFS patients that are understood to derive benefit from ganciclovir treatment. Our. . .

DETD [0150] Results of Copalis.RTM. Assays Specificity: The serum of 17 patients were studied who had no cytomegalovirus IgG or IgM ***antibody*** to structural viral proteins as measured by the classical ELISA method. In these 17 patients there were no ***antibodies*** by the Copalis.RTM. method using light scattering technique to either viral protein (VP), CM.sub.2 or p52. Therefore, no false-positives by. . .

DETD . . . the 121 separate patients were similarly tested. These patients were positive by the ELISA classical technique for structural cytomegalovirus IgG ***antibody***. They were negative for cytomegalovirus IgM ***antibody***. All 121 of these patients positive for cytomegalovirus IgG ***antibody*** were positive for viral protein (Copalis.RTM.). The antigen for the cytomegalovirus ELISA technique is nonpurified, human fiberblast tissue culture suspension. . . cases which were positive for cytomegalovirus IgG by both Copalis.RTM. and ELISA methods, they were negative by Copalis.RTM. method for ***antibodies*** to p52 and CM.sub.2. None of these patients had the chronic fatigue syndrome. There was one patient who was positive in very low titer to cytomegalovirus IgG by the Copalis.RTM. method which was negative for IgG ***antibody*** to cytomegalovirus by the ELISA method. These data indicate that Copalis.RTM. light scatter

antibodies do not occur with nonstructural proteins CM.sub.2 or p52 in patients with past cytomegalovirus infection. Seventy-six sera were analyzed which were positive for viral capsid antigen IgM to Epstein-Barr virus and/or EA ***antibody*** to Epstein-Barr virus. These cases had no cytomegalovirus ELISA IgM or IgG. The obtained serum in 76 cases was uniformly negative by Copalis.RTM. tests to human cytomegalovirus IgG and IgM. The IgM includes both nonstructural ***antibodies*** CM.sub.2 and p52. Therefore Epstein-Barr virus did not cause false-positive Copalis.RTM. results. The tests by the Copalis.RTM. method are greater. . .

DETD [0152] Results of Copalis.RTM. ***antibody*** testing in patients with the U.S. CDC-Chronic Fatigue Syndrome and various other patients with severe onset of explained fatigue. Group. . . chronic fatigue syndrome. They are six women and eight men with ages ranging from 32 through 48. They had IgG ***antibodies*** to human cytomegalovirus. They had no IgM ***antibodies*** to human cytomegalovirus by the ELISA technique. They would be evaluated as having past infection. These eight patients had high titer positive ***antibodies*** to nonstructural gene products p52 and CM.sub.2. They were treated with intravenous ganciclovir and followed by oral ganciclovir. These nonstructural ***antibodies*** became markedly lower and disappeared. Moreover, the fatigue in these patients was remarkably lessened. The use of the Copalis.RTM. ***antibody*** titer in these patients depicted incomplete virus multiplication and the reversal of this immune status associated with the improvement of. . . a 27 year old woman and a 48 year old woman had similar evidence of chronic fatigue syndrome and high ***antibody*** titers to the nonstructural gene products of human cytomegalovirus p52 and CM.sub.2. These patients could not be treated with anti-viral agent, ganciclovir. Nevertheless, their initial diagnosis was secured by the presence of the ***antibodies*** to p52 and CM.sub.2.

DETD [0153] FIG. 4 is a table depicting the ***antibody*** titer levels of one of the patients, a 47-year old male, before and after antiviral therapy. This patient had IgG ***antibodies*** to HCMV but no IgM ***antibodies*** by the ELISA technique. As shown, this patient had high titer positive ***antibodies*** to nonstructural gene products p52 and CM.sub.2. After antiviral treatment, these nonstructural ***antibodies*** became markedly lower.

DETD . . . with the chronic fatigue syndrome are of interest. In two of these patients, the patient had, of course, IgG ELISA ***antibodies*** to human cytomegalovirus and no IgM ***antibodies*** to the ELISA testing. They would be ordinarily read as having past infection. They had, however, markedly high ***antibody*** titers to CM.sub.2. They had no elevated ***antibody*** titers to p52. They were treated with intravenous ganciclovir followed by oral ganciclovir. The CM.sub.2 ***antibodies*** lessened or disappeared and they improved. The third patient, a 39 year old woman, with chronic fatigue syndrome was diagnosed. . . This patient was similarly treated with intravenous ganciclovir followed by oral ganciclovir and improved remarkably. Therefore, the decreasing response to ***antibodies*** to HCMV nonstructural gene products (CM.sub.2) after specific antiviral administration defines human cytomegalovirus chronic fatigue syndrome and define three kinds. . . Several other patients who had the sudden onset of ongoing, unexplained fatigue also have been studied and found to have ***antibodies*** to the p52 and CM.sub.2. One of these was

an 83 year old woman with congestive heart failure and diabetes. . . after intravenous ganciclovir. A second patient is a 75 year old woman with fatigue. Both of these older women had ***antibody*** responses to both CM.sub.2 and p52. A 60 year old man with morbid obesity and diabetes mellitus suddenly became fatigued. . . fatigue. He also was identified as having incomplete cytomegalovirus infection by the presence of high titers of the two nonstructural ***antibodies***. Finally, an 82 year old woman had ongoing fatigue of unknown cause. She had ***antibodies*** only to the p52 epitopes.

DETD . . . depicted in FIG. 3, the administration of antiviral therapy, specifically both ganciclovir and valacyclovir, remarkably reduced the presence of high ***antibody*** titers to both HCMV and EBV.

DETD . . . invention provides an exact means of diagnosing a subset of patients with cytomegalovirus chronic fatigue syndrome. The decrease in these ***antibody*** titers has been identified and the use of specific anti-viral therapy has resulted in significant patient improvement, accompanied by a . . .

CLM What is claimed is:

. . . for serologic evidence of EBV and HCMV, further comprising:
obtaining serum from the patient; measuring the level of EBV IgM ***antibodies*** to the VCA in the serum; measuring the level of EBV ***antibodies*** to the total EA in the serum; measuring the level of HCMV IgM ***antibodies*** in the serum by measuring antigens p52 and CM.sub.2 with the use of a light scattering technique; measuring the level of HCMV IgG ***antibodies*** in the serum by measuring antigens p52 and CM.sub.2 with the use of a light scattering technique; monitoring the patient. . . of the chronic fatigue syndrome when the measurements show any one of the following: 1) an elevated level of IgM ***antibodies*** to the VCA for EBV; and 2) presence of total EA ***antibodies*** for EBV, in combination with the absence of IgM ***antibodies*** for HCMV and a low level of IgG ***antibodies*** for HCMV; classifying HCMV as the cause of the chronic fatigue syndrome when the measurements show any one of the following: 1) an elevated level of IgM ***antibodies*** for HCMV; and 2) an elevated level of IgG ***antibodies*** for HCMV, in combination with a low level of IgM ***antibodies*** to the VCA for EBV, and the absence of total EA ***antibodies*** for EBV; classifying a combination of EBV and HCMV as the cause of the chronic fatigue syndrome when the measurements show any one of the following: 1) an elevated level of IgM ***antibodies*** to the VCA for EBV; and 2) the presence of total EA ***antibodies*** for EBV, in combination with any of the following: 1) an elevated level of IgM ***antibodies*** for HCMV; and 2) an elevated level of IgG ***antibodies*** for HCMV; administering to the patient a therapeutically effective amount of one or more pharmaceutically acceptable antiviral agents suitable for. . .